THE INFLUENCE OF THE RENIN-ANGIOTENSIN SYSTEM ON THE RELEASE OF CATECHOLAMINES FROM THE ADRENAL MEDULLA

BY

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Declaration

This thesis is entirely my own composition and the work detailed within is my own except where indicated.

SIGNED

ABSTRACT

Anaesthetised cats and dogs were used to show:-

1. The angiotensin converting enzyme inhibitor, captopril, inhibited the basal output of catecholamines from the adrenal medulla and the reflex release induced by a lowering of carotid perfusion pressure (baroreceptor test).

2. Plasma renin activity did not increase during the ten minute baroreceptor tests.

3. A non-pressor level of angiotensin II (AII) reversed the inhibitory effects of captopril. These results suggest that a minimum circulating level of AII is required for the adrenal gland to respond to the reflex stimuli, and AII exerts a direct facilitatory effect on the adrenal gland.

4. Captopril reduced resting systemic blood pressure and the reflex pressor response to baroreceptor tests.

Anaesthetised dogs were used to show:-

5. Captopril increased adrenal blood flow, an effect probably partially related to a reduction in haematocrit.

6. Coadministration of captopril and cycloheximide, which inhibits corticosteroid secretion in response to adrenocorticotrophic hormone

(ACTH), severely inhibited resting adrenal catecholamine release and abolished the reflex release in response to baroreceptor tests, and this effect was not overcome by administration of AII. ACTH reversed the inhibitory effects of captopril on adrenal catecholamine release, and did not increase the ratio of adrenaline : noradrenaline in adrenal venous blood.

7. Captopril reduced adrenocorticosteroid concentration in adrenal venous blood. Adrenocorticosteroids have previously been shown to facilitate adrenal catecholamine release. The results suggest that AII may facilitate adrenal catecholamine release partially by an indirect facilitatory effect on adrenocorticosteroid secretion.

8. Captopril inhibited, and a non-pressor level of AII restored, the adrenal release of catecholamines evoked by splanchnic nerve stimulation, from the decentralised adrenal gland. This suggests that AII may facilitate splanchnic nerve activity and that the facilitatory effect of AII is at the level of the adrenal gland and not at the level of the central nervous system. The AII antagonist, saralasin, also inhibited the adrenal release of catecholamines in response to splanchnic nerve stimulation, from the denervated adrenal gland.

Introductory experiments were performed on anaesthetised dogs to show the following:-

9. The opiate antagonist, naloxone, increased resting adrenal catecholamine release and both reflex release from the innervated gland and splanchnic nerve stimulation-induced release from the

denervated gland. This suggests that opioid peptides, stored in the adrenal medullae and splanchnic nerves, inhibit adrenal catecholamine release. Captopril inhibits the breakdown of opioid peptides and this may contribute to its inhibitory effect on adrenal catecholamine release.

10. The cyclo-oxygenase inhibitor, indomethacin, inhibited both reflex catecholamine release from the innervated adrenal gland and splanchnic nerve stimulation-induced release from the denervated gland, and thus potentiates the inhibitory effects of captopril. This suggests that prostaglandins facilitate adrenal catecholamine release. Captopril has been shown to stimulate prostaglandin synthesis, but indomethacin did not blunt the hypotensive effect of captopril.

11. Indomethacin reduced adrenal blood flow and induced a vasoconstriction in the adrenal vascular bed. This suggests that prostaglandins may play a role in the maintainance of adrenal blood flow through a tonic vasodilation.

12. The hypotensive effect of captopril and its inhibitory effects on adrenal catecholamine release, were not attenuated by depressed plasma renin activity.

CONTENTS

Introduction

General outline of research

I shall outline here the reasons for doing this research project and the main incentives behind the progress of the research. For a detailed review of literature and outline of each step taken, I refer the reader to the "Introduction and literature review" of Parts 1-5 of this thesis. I shall refer to the relevent sections during this general outline.

In 1977, Feuerstein, Boonyaviroj and Gutman reported that, in the anaesthetised cat, haemorrhage induced a reflex release of catecholamines from the adrenal medulla. They observed that blockade of the renin-angiotensin (AII) system either by bilateral nephrectomy or by administration of the AII antagonist saralasin (see "An introduction to the renin-AII system and angiotensin- converting enzyme inhibitors") inhibited this reflex release of catecholamines. They also observed an increase of circulating plasma renin activity (PRA) following haemorrhage. They concluded that the reflex release of catecholamines was due to an increase in circulating AII synthesis, initiated by haemhorrhage, acting centrally to increase sympathetic drive. Such an increase in sympathetic drive would stimulate catecholamine release from the adrenal medulla,. mediated by splanchnic nerve stimulation.

Injury, dehydration, exertion and other stresses to the cardiovascular system are also known to induce a release of catecholamines from the adrenal medulla. Drugs which inhibit the renin-

AII system are now widely used in the treatment of hypertension (see "Part 1 - Introduction and literature review"). We considered it important to understand the extent to which such drugs, in particular the angiotensin- converting enzyme inhibitor (ACEI) captopril, impair this protective physiological response to cardiovascular stress. Such an understanding would assist in the clinical prediction and management of the impaired responses to cardiovascular stress in patients receiving drugs that inhibit the renin-AII system.

AII is known to stimulate release of catecholamines from the adrenal medulla directly, (see "Part 1") and we thought it possible that the results of Feuerstein etal could be reinterpretated to suggest that AII was acting directly on the adrenal medulla, and not via the central nervous system as they suggested. We thought it possible that a minimum level of circulating AII was required for the adrenal medulla to respond to such stimuli.

Our initial experiments were on anaesthetised cats in order to compare our results with those of Feuerstein etal (1977). Subsequent experiments were carried out on anaesthetised dogs due to the greater reproducebility and success of our experiments in the dog.

Haemorrhage would induce a lowering of carotid perfusion pressure. Instead of inducing haemorrhage, we chose to induce a direct lowering of carotid perfusion pressure (I shall refer to this as "baroreceptor stimulation or baroreceptor test" (see "The arterial baroreceptor")), in order to stimulate a reflex release of catecholamines from the adrenal medulla. This afforded us a greater

control over experimental conditions.

We discovered that captopril did indeed inhibit the release of catecholamines in response to baroreceptor stimulation, but within the time course of our ten minute baroreceptor tests there was no evidence of an increase of circulating PRA. This did suggest that a minimum circulating level of AII was required for the adrenal gland to respond to such reflex stimuli and when this was removed by captopril, the adrenal medullary response was inhibited. The resting release of catecholamines, prior to baroreceptor stimulation was also inhibited by captopril, and both the resting release and reflex release of catecholamines could be restored by an exogenous infusion of a non-pressor level of AII.

Adrenocorticosteroids can regulate the response of the adrenal medulla to hypotension, and AII can stimulate adrenocorticosteroid secretion from the adrenal cortex. We thought it possible that part of the facilitatory effect of AII on catecholamine release could be due to an effect on adrenocorticosteroid secretion which may facilitate the release of both adrenaline and noradrenaline from the adrenal medulla (see "Part 2").

We investigated this possibility and discovered that cycloheximide, a drug known to inhibit adrenocorticosteroid secretion in response to adrenocorticotrophic hormone (ACTH), inhibited both resting and reflex adrenomedullary catecholamine release, and prevented restoration of the adrenal response by AII. ACTH restored the release of catecholamines previously inhibited by captopril. We

also determined the effect of captopril on cortisol and corticosterone output from the adrenal gland, and discovered that captopril reduced the adrenal venous concentration of both adrenocorticosteroids. This evidence supported our theory that AII may affect catecholamine release indirectly through facilitating adrenocorticosteroid secretion.

Although our results did suggest that AII was exerting a direct facilitatory effect on catecholamine release from the adrenal medulla, we still could not exclude the possibility that the effect was due to central stimulation of sympathetic drive. We therefore investigated whether captopril could inhibit the release of catecholamines from the denervated adrenal gland. We also wanted to investigate whether AII potentiated catecholamine release in response to splanchnic nerve stimulation. This seemed possible as there is much evidence to suggest a facilitatory role for AII on stimulation-evoked release of noradrenaline from most sympathetic nerves and also on stimulation-evoked acetylcholine release from both sympathetic and parasympathetic ganglia (see Part 3).

We discovered that captopril inhibited both the resting release of catecholamines from the denervated adrenal gland and the release evoked by splanchnic nerve stimulation. These releases could be restored by an infusion of a non-pressor level of AII. These results supported a direct facilitatory effect of AII on catecholamine release from the adrenal medulla. They also supported a facilitatory effect effect of AII on splanchnic nerve stimulation-induced catecholamine release.

We had answered the main questions that this research had set out to investigate at this point. Due to having only a limited ammount of research funding remaining, I had to choose between investigating one more area of interest in detail, or carrying out some introductory experiments in two areas. I chose the latter option, as the two areas were of equal interest to me. As the numbers of dogs available was limited, the experiments described in parts 4 and 5 should be considered as introductory, and additional to the main core of this research project.

The first area of interest arose due to available evidence which suggested that captopril inhibits the carboxypeptidase enzyme responsible for the breakdown of endogenous opioid peptides. Opioid peptides are abundant in both the adrenal medulla and the splanchnic nerve. There was evidence to suggest that opioid peptides may inhibit release of catecholamines from the adrenal medulla. It was possible that the prevention of opioid peptide degradation by captopril could contribute to its inhibitory effect on catecholamine release from the adrenal medulla. I investigated this possibility (see "Part 4"). During this series of experiments I also investigated the effect of dietary sodium on the resposes of the adrenal medulla to captopril, in order to investigate whether PRA was related to the effectiveness of captopril in the anaesthetised dog.

The second arose due to available evidence which suggested that captopril increases secretion of prostaglandins from many sites. I investigated the possibility of an interaction between captopril and prostaglandin production on the release of catecholamines from the

adrenal medulla (see "Part 5").

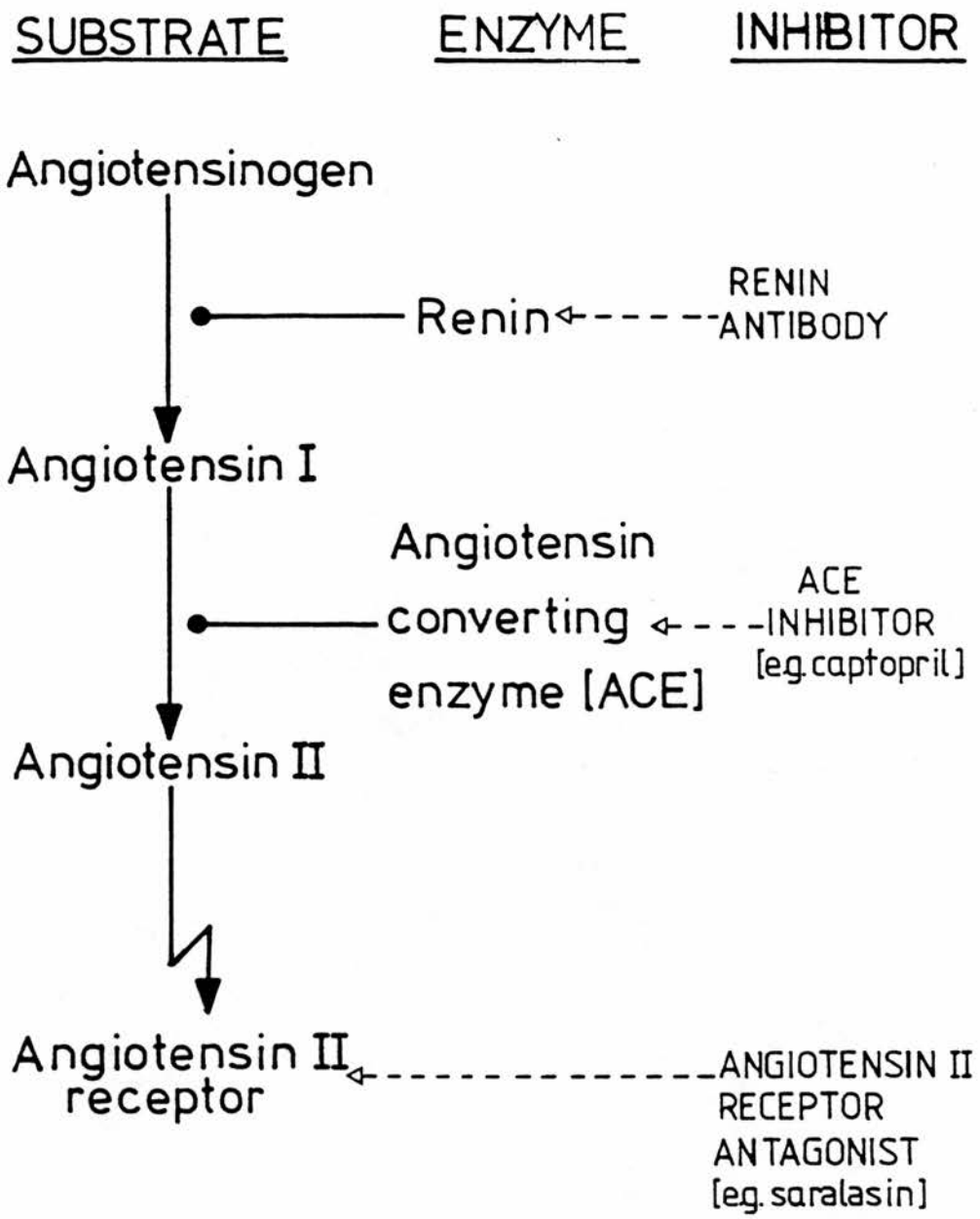
While emphasis has been placed on catecholamine release from the adrenal medulla , it was also possible to determine the effects of captopril, saralasin, AII, adrenocorticosteroids, cycloheximide, naloxone and indomethacin on other physiological aspects such as blood pressure and adrenal blood flow.

An introduction to the renin-angiotensin system and angiotensin-converting enzyme inhibition.

Renin is predominantly synthesised within the juxtaglomerular apparatus, lining the arterioles of the kidney. It is a proteolytic enzyme which cleaves angiotensinogen, a plasma globulin synthesized in the liver, to produce the decapeptide angiotensin I. Angiotensinogen is always present in the blood and it is the rate of secretion of renin in the kidney which is the rate- limiting step in angiotensin synthesis. Renal sympathetic nerves and circulating adrenaline are two primary stimulants for renin release. A reduction in body sodium levels and extracellular volume, detected by the chemoreceptors and baroreceptors, trigger a reflex increase in sympathetic tone to the kidneys, promoting renin release.

Angiotensin I is converted to the octapeptide Angiotensin II (AII) by the angiotensin-converting enzyme (ACE), located primarily within the pulmonary circulation. AII stimulates vascular smooth muscle and is a potent vasoconstrictor. It also stimulates catecholamine secretion by the adrenal medulla and is the primary input into the adrenal cortex, promoting synthesis and secretion of aldosterone and therfore sodium retention. Both vasoconstriction and sodium retention can increase blood pressure and much attention has been directed towards developing drugs which prevent the actions of AII, for the treatment of renin-dependant hypertension.

There are three major ways by which drugs could inhibit the renin-AII system (see Figure M1)-:



The synthetic pathway for angiotensin II indicating the three sites of action for inhibitors of the renin-angiotensin system.

Figure M1

1. Inhibition of the renin-angiotensinogen reaction.

2. Blockade of the AII receptor.

3. Inhibition of ACE.

1. Renin antibodies have been raised against the catalytic site of the enzyme and have been used to investigate the role of renin in the control of homeostasis and experimental models of hypertension (Haber, Dzau, Kopelman, Slater and Berger, 1980). Monoclonal, antihuman renin-specific antibodies have been isolated using cell fusion techniques and could become useful drugs for the treatment of malignant hypertension (Dzau, Devine, Mudgett-Hunter, Kopelman, Berger and Haber, 1983).

Renin normally cleaves a leucine-leucine bond in the angiotensinogen molecule. Burton, Cody, Herd and Haber (1980) reported that if phenylalanine is substituted for leucine , the resulting peptide serves as a poor substrate for renin. It is a competitive inhibitor for the reaction of renin with angiotensinogen. In addition, if a proline residue was added at the amino terminus and a lysine residue added at the carboxy terminus of this molecule, the resulting peptide inhibited the action of renin in vivo.

Such inhibitory peptides are unlikely to become clinically useful as they have many other actions besides inhibiting peripheral renin activity. In particular, evidence indicates that they produce parasympathetic stimulation resulting in sinus bradycardia, venous dilation and a fall in cardiac output (Zusman, 1984).

2. The substitution of sarcosine and alanine for the naturally occuring amino acids at positions one and eight in the AII molecule yields the highly competitive AII receptor antagonist, saralasin (Khosla, Smeby and Bumpers, 1974). This has a hypotensive effect in patients with high-renin related hypertension and in experimental models of renovascular hypertension. In low-renin states, however, it exerts AII agonistic activity and increases blood pressure (Pals, Masucci, Sipos and Dennings, 1971). This, and the need for saralasin to be administered parenterally, limit its clinical usefulness.

3. In 1968 Bakhle reported that a mixture of polypeptides isolated from the venom of the snake "Boyhrops jajaraca" inhibited the conversion of angiotensin I to AII, and also potentiated the effects of bradykinin. Ferreira etal purified individual peptides from the snake venom and demonstrated that these inhibited ACE and bradykinase in pulmonary homogenate (Ferreira, Greene, Alabaster, Bakhle and Vane, 1970; Ferreira, Bartelt and Greene, 1970).

The first ACE inhibitor studied in man was SQ 20881,or teprotide, a synthetic nonapeptide. This reduced blood pressure in patients with high plasma renin activity, but because of its short duration of action and the need for parenteral administration, its clinical usefulness was limited (Antonaccio, 1982).

ACE was discovered to be a zinc-containing metalloprotein similar to pancreatic carboxypeptidase A (Cushman, Cheung, Sabo and Ondetti, 1978). Cushman, Cheung, Sabo and Ondetti (1977) sequentially analysed compounds known to inhibit carboxypeptidase A and discovered that an

analogue of proline, D-3- mercapto-2- methyl-propanoyl-1-proline, was a highly specific inhibitor of ACE in vitro. The alpha-methyl group and the carboxyl groups on proline were later shown to interact with the hydrophobic sites of ACE. The mercapto substitution on the beta-carbon promoted a specific interaction with the zinc atom in ACE and this accounts for the potent competitive inhibitory properties of the molecule (Cushman and Ondetti, 1980). This molecule was SQ 14225 or captopril and is an orally active potent inhibitor of ACE activity. It is now extensively used for the treatment of hypertension.

Other ACE inhibitors have since been developed, (eg: Enalapril (Bauer, 1984)) with similar pharmacological profiles to captopril (Schiffrin, Gutkowska, Thibault and Genest, 1983).

The adrenal gland

I shall give a very brief description of the anatomy, blood supply and innervation of the adrenal gland, covering those points I consider relevant to this research and experimental procedures. For a detailed review of the adrenal gland, I refer the reader to "Handbook of Physiology", Endocrinology VI, 1975, Chapters 22-31."

1. Anatomy.

The mammalian adrenal gland derives its name from its position on the anterior surface of the kidney and in all but the primate, they lie seperate from the kidney. The left adrenal gland lies between the medial border of the superior half of the left kidney and the aorta, while the right adrenal gland lies between the liver and the vena cava. Both lie on the dorsal abdominal wall. The left adrenal gland is more accessible for dissection and cannulation and is therefore the one chosen by us for study in the anaesthetised cat and dog.

The adrenal gland is composed of an outer cortex and an inner medulla, bound by a thin capsule.

The cortex is developed from the cells of the coelomic mesothelium and is composed of three functionally separate zones. The outermost region of the mammalian adrenal (just below the gland capsule) is called the zona glomerulosa and is the site for synthesis of mineralocorticoids, desoxycorticosterone and aldosterone. Below the zona glomerulosa is the zona fasciculata which is usually the largest

part of the adrenal cortex and is the principle site for synthesis and secretion of glucocorticoids. The innermost zone, closest to the adrenal medulla, is the zona reticularis and it secretes glucocorticoids and sex steroids. The secretions of the zonae fasciculata and reticularis are closely controlled by adrenocorticotrophic hormone, produced by the pituitary, which in turn is controlled by corticotrophin releasing factor, produced by the hypothalamus.

The medulla is developed from ectodermal cells derived from the neural crest and is considered to be an enlarged, highly specialized sympathetic ganglion. It is composed of large polygonal cells separated by numerous sinusoidal vessels. Adrenal medullary cells stain brown with fixatives containing chromium salts and are often referred to as chromaffin cells or phaeochromocytes. These chromaffin cells are granular, the granules evenly distributed in the cytoplasm. They synthesise, store and release adrenaline and noradrenaline.

2. Blood supply

Numerous arteries approach the adrenal gland, derived from the aorta, phrenic, renal, adrenolumbar, coeliac and superior mesenteric arteries. They form a capsular plexus in the adrenal gland. The majority of arterial branches that penetrate the capsule divide into capillaries that pass between and around the cells of the zona glomerulosa, between the cells of the zona fasciculata and anastamose with each other. Two types of small arteries have also been identified, one terminating in the adrenal cortex and one passing

straight to the adrenal medulla where they terminate in the adrenal medullary capillary plexus. The capillary plexus of the cortex forms cortical venous sinuses with the medullary plexus. Blood passing through these sinuses comes into intimate contact with chromaffin cells. Two to four veins leave each adrenal medulla to the adrenolumbar vein and small venous channels connect on the venous side of the capsular plexus to the veins which accompany the adrenal arteries and return to the renal, phrenic and adrenolumbar veins. In the dog and cat the adrenolumbar vein fuses with either the vena cava or the renal vein a short distance from the adrenal gland. It is this short vessel between the adrenal gland and vena cava that I refer to as the adrenal vein in subsequent sections of this thesis.

3. Innervation of the adrenal medulla

As previously mentioned the adrenal medulla is recognised as a specialised sympathetic ganglion. As such, the main innervation to the adrenal gland is cholinergic and is from the greater splanchnic nerve, which normally leaves the sympathetic trunk at the 12th thoracic sympathetic ganglion. The lesser splanchnic nerves (normally two) usually leave at the 1st lumbar and 13th thoracic sympathetic ganglia and provide a minor contribution to the innervation of the adrenal medulla. All splanchnic nerve fibres fan out into many branches as they approach the dorsum of the adrenal gland.

The adrenal cortex has no nerve supply, except to its blood vessels.

The arterial baroreceptor

I shall briefly describe the location and function of the arterial baroreceptors, covering those points relevant to our experimental procedures.

Arterial baroreceptors are located at the carotid sinus bifurcations, aortic arch, thyrocarotid junction and cardiopulmonary area. The baroreceptors are specialised stretch receptors responding to expansion of the vessel wall. In the "Methods" section I shall describe the proceedure used for stimulation of the baroreceptors located at the carotid sinus in detail. Stimulation of the arterial baroreceptor by a decrease in carotid perfusion pressure reduces a tonic inhibitory influence which normally presides on sympathetic drive to the heart, blood vessels and adrenal medulla. This results in an increase in sympathetic discharge and is the means by which we stimulate a reflex release of adrenomedullary catecholamines in our experimental animals. I subsequently refer to this procedure as "baroreceptor stimulation".

Methods

An introduction to catecholamine assay

Several methods have been described to determine the content of adrenaline and noradrenaline in plasma, urine, cerebrospinal fluid and tissues. The main techniniques available are-:

1. Biological assay - dependant on the pharmacological activity of adrenaline and noradrenaline (see Gaddum, 1959; Callingham, 1967 for reviews).

2. Chemical techniques - discussed later (see Udenfriend, 1962; Callingham, 1967 for reviews).

3. Radioenzymatic proceedures and double isotope derivative analysis - The radioenzymatic proceedures employ the specific radiolabelling of the catechol moiety with 5-adenosylmethionine and catechol-O-methyl transferase (Ben-Jonathon and Porter, 1976). They require multiple sample handling steps, labelled compounds and enzyme preparations. This makes them too time consuming and expensive for routine catecholamine analysis. Double isotope derivative analysis (Engelman, Portnoy and Lovenberg, 1968) has similar disadvantages.

4. Gas Chromatography - This requires that catecholamines are treated with halogenated anhydrides which gives volatile derivatives which possess electron-capturing properties (Gelpi, Peralta and Segura, 1974). Sensitivity is often reduced due to the sample background arising from derivatization reagents and contaminants from the sample matrix and solvents. This, together with the high cost instrumentation

required makes this method unsuitable for routine catecholamine analysis.

5. Radioimmunoassay - This employs standard radioimmunoassay proceedures (Johnson, Kupiecki and Baker, 1980). It is extremely sensitive but too expensive for routine analysis.

6. High Performance Liquid Chromatography (HPLC) - discussed later (see Davis, Schoemaker, Chen and Yamamura, 1982; Krstulovic, 1982; Hjemdahl, 1984).

The two techniques I chose to use were the chemical technique dependant on the formation of fluorescent derivatives of the catecholamines, using spectrofluorimetric estimation and, subsequently, HPLC with electrochemical detection.

Chemical spectrofluorimetric catecholamine assay (CSCA) was initially the method of choice as it fulfilled the criteria required of an assay system for catecholamine content in adrenal venous blood.

Adrenal venous blood contains very high concentrations of catecholamines and therefore a very high sensitivity (as obtained by radioimmunoassay) was not required. A simple extraction and assay procedure which yielded consistent results was required. CSCA provided a technique which was simpler than biological assay and isotope derivative techniques. It provided consistent results and was relatively inexpensive to run in comparison with radioimmunoassay. CSCA also allowed an adequate differentiation between adrenaline and

noradrenaline and allowed a high throughput of samples, which was necessary as many adrenal blood samples were collected during each experiment.

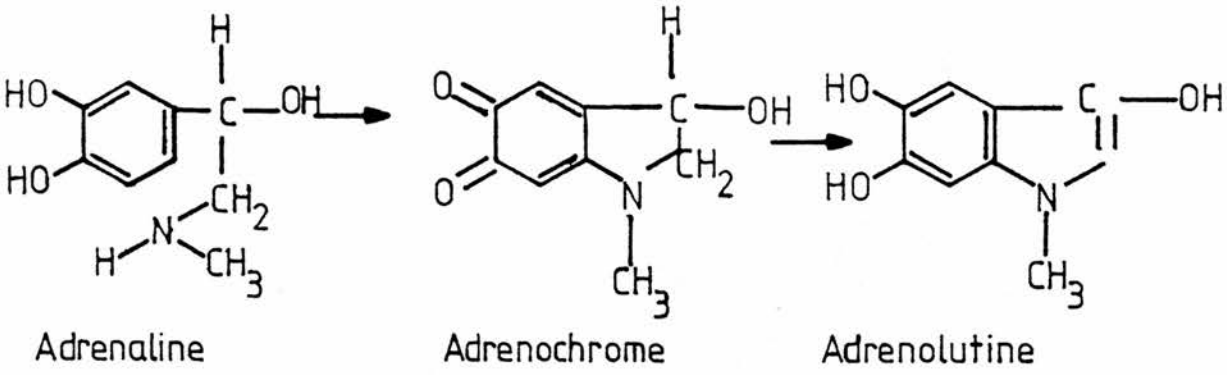
My reasons for changing to HPLC for determination of catecholamines are discussed later.

Chemical specrofluorimetric catecholamine assay

Introduction

Chemical specrofluorimetric catecholamine assay (CSCA) depends on the observation that adrenaline, noradrenaline and their oxidation products fluoresce. This was first observed by Vulpian in 1856 and in 1918 Loew observed that in strong alkali solution adrenaline produces a yellow- green fluorescence. Gaddum and Schild (1934) first attempted to develop an assay for catecholamines (CAs) based on this chemical reaction. They also demonstrated that the reaction required the presence of oxygen. Their assay was unsuccessful due to the fluorescence being transient. Noradrenaline also gave a much weaker fluorescence than adrenaline. In 1948, Ehrlen overcame the problem of stability by discovering that ascorbic acid stabilised the adrenaline fluorophor. The first successful fluorimetric assay was subsequently developed by Lund in 1950.

The critical reaction is illustrated below-:



Adrenaline and noradrenaline are oxidised to the cyclised coloured adrenochrome and noradrenochrome respectively (Richter and Blaschko, 1937). Addition of alkali isomerically transforms these "chrome" derivatives to form "lutines" (Lund, 1950). The fluorescent derivative of adrenaline was identified as 1- methyl-3,5,6-trihydroxyindole (adrenolutine), and the reaction became known as the "trihydroxyindole" (THI) reaction.

The oxidation rates of adrenaline and noradrenaline are pH dependant and it was this observation that Lund (1950) used to develop a differential assay for the two catecholamines. This technique was later improved by Price and Price in 1957. Instead of using oxidation at two pH levels they used light at two wavelengths to differentiate between adrenolutine and noradrenolutine. They also introduced potassium ferricyanide as the oxidising agent. Previously, manganese dioxide and iodine had been used. Potassium ferricyanide yields a greater fluorescence and reduces interferance from dopamine, Dopa (Valori, Brunori, Renzini and Corea, 1970) and laboratory lighting.

They also increased the stability and intensity of the fluorescence by reacidifying the reaction products from the alkaline reaction to pH 5.0.

Many variations of the CSCA have developed, differing primarily in the choice of oxidising and stabilising reagent. (For reviews see Udenfriend, 1962; Haggendal, 1966; Callingham, 1967).

The observation by Vendsalu in 1960 that ascorbic acid itself gives rise to fluorescent oxidation products when in alkaline solution induced the introduction of sulphur containing compounds as stabilising reagents. These include British Anti-Lewisite (BAL)/formaldehyde mixture (Valori etal, 1970), BAL/sodium sulphite mixture (Haggendal, 1966), sodium sulphite (Laverty and Taylor, 1968), cysteine hydrochloride (Klensch, 1966) and thioglycolic acid (Merrills, 1963).

The actual method I have employed is a modification of the techniques described by Haggendal in 1966 and Valori etal in 1970.

Extraction of catecholamines from plasma, and proceedures for CSCA

Blood plasma must be purified before CSCA can be applied, to minimise the occurrance of contaminants which could affect the detection of fluorescence.

The method I initially chose was cation exchange chromatography as it lent itself well to CSCA, in particular the system developed in

this laboratory. I later changed to alumina adsorption when I changed my method of catecholamine detection to HPLC. I shall discuss this later in "HPLC with electrochemical detection for the assay of catecholamines".

The extraction of catecholamines using cation exchange chromatography depends on the catecholamines being positively charged around a neutral pH. This results in them being retained by negatively charged groups on either sulphonic acid or carboxylic acid resins. The catecholamines are then readily eluted off the resin by an acidic solution.

1. Collection of adrenal venous blood

Venous blood from the left adrenal gland was collected in cooled, graduated centrifuge tubes (see "Methods"-Whole animal experiments).

2. Separation and storage of plasma

Blood samples were centrifuged at 3,000 r.p.m., 4°C for ten minutes. The total blood volume, red blood cell volume and plasma volume were recorded and the plasma removed to fresh storage tubes, using a pasteur pipette. A solution of $2mgml^{-1}$ sodium metabisulphite was prepared and a volume equal to that of the plasma added, ensuring $2mgml^{-1}$ plasma was added to each sample. (Sodium metabisulphate is an antioxidant used to minimise oxidation of the catecholamines during storage, defrosting etc..) The samples were frozen overnight at -20°C.

3. Extraction of catecholamines

The plasma samples were allowed to thaw. Any fibrin present was removed by rotating a fine glass rod slowly around each sample. The fibrin attached to the rod and was removed.

Duplicate aliquots of each plasma sample were then loaded onto Amberlite CG50 (mesh size 100-200) resin columns of 5cms length and 0.25 cm internal diameter. (See section on "Preparation of Amberlite CG50 columns" later, for details of CG50 resin and the preparation of the columns used).

The columns used consisted of a 20ml reservoir leading down to the 0.25 cm internal diameter glass column. The samples were held by the reservoir until they were induced to pass down the column, by a fine glass rod.

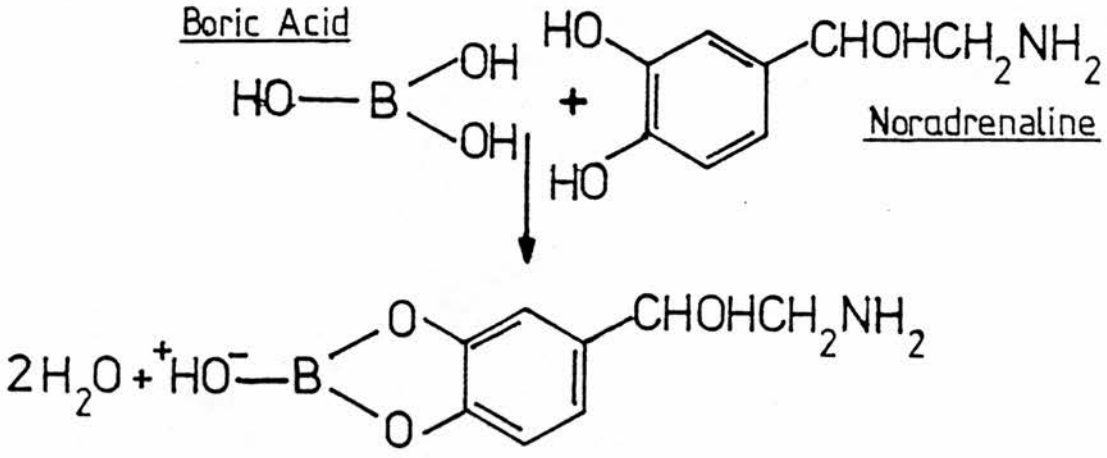
To each plasma sample 0.1N phosphate buffer pH 7.4, (447mg monopotassium dihydrogen orthophosphate, 2.383g disodium hydrogen orthophoshate in 250ml distilled water) and 3% EDTA was added in the ratio 1:3:0.5, plasma:buffer:EDTA respectively. The samples were then induced to run through the column using a fine glass rod.

The columns were then rinsed with 5mls of distilled water.

The catecholamines were eluted from the columns with 1ml of 2/3M boric acid, into test-tubes selected to fit both the AC60 autoanalyser and the spectrophotofluorimeter.

A volume of 1ml 2/3M boric acid completely elutes the catecholamines and is the ideal volume required for the CSCA. 2/3M boric acid is used, as boric acid is insoluble at room temperature at higher concentrations. Adrenaline and noradrenaline form complexes with boric acid (Trautner and Messer, 1952) which promotes elution. These complexes are stable at pH 6.5, enabling the eluates to be stored for up to three hours.

Formation of borate-noradrenaline complex



Column standards - 0.1 ml of 1μgml^{-1} standard solutions of adrenaline and noradrenaline were loaded onto the columns in 3ml 0.1N phosphate buffer (pH 7.4) and 0.05 ml of 3% EDTA.

Non-column blanks - these were 1ml aliquots of 0.4N phosphate buffer, pH6.5.

Non-column standards - these were 0.1ml of 1μgml standard solutions of adrenaline and noradrenaline and 0.9ml of 0.4N phosphate

buffer, pH 6.5 (6.805g monopotassium dihydrogen orthophosphate and 2.363g disodium hydrogen orthophosphate in 1L distilled water).

4. Spectrophotofluorimetric assay

4.1.Preparation of fluorophors

The maximum number of samples assayed at any time was 12, assayed in duplicate, due to availability of the columns.

The tubes were then placed into the conveyor belt of a unicam AC60 autoanalyser in the following order-:

1+2 Non-column adrenaline standard
3+4 Non-column noradrenaline standard
5+6 Non-column blank
7+8 Column adrenaline standard
9+10 Column noradrenaline standard
11+12 Column blank
13-24 Samples 1-6 in duplicate
25-36 As 1-12
37-48 Samples 7-12 in duplicate

The THI reaction was then induced in each sample. The following reagents were added to each tube and mixed-:

Time (minutes)	Volume (ml)	Reagent
0	0.5	Potassium ferricyanide (0.05%) containing cupric chloride (0.00025%)
3	0.5	Sodium sulphite (10%) containing B.A.L (0.4%)
3.5	0.5	Sodium hydroxide (8N)
6.5	0.35	Glacial acetic acid (conc.)

The final pH of each sample was 5.0. The samples were then removed from the autoanalyser, the outside of each tube carefully wiped, and each allowed to stabalise for 30 minutes before being transferred to the Aminco-Bowman spectrophotofluorimeter.

4.2. Reading of fluorescence

The excitation spectrum scanned over the range 300-500mμ and the emission wavelength was set at 510 mμ. The Aminco-Bowman Photomultiplier Microphotometer displayed the intensity of the fluorescence in each sample. The intensity was also displayed on a "servoscribe" flat-bed recorder, the chart drive of which was synchronised to the scanning motor switch on the spectrofluorimeter and operated only when the excitation spectrum was scanned. The chart and wavelength scanning motor speeds were fixed and the wavelength at any point on the trace was found by its distance from the start of each scan. For each sample a maximum fluorescence peak was obtained on the trace by adjusting the meter multiplier switch on the photomultiplier accordingly.

Wavelength selection

The "index of discrimination" (I.O.D.) was calculated for each sample, ie-:

$$\text{I.O.D.} = \frac{\text{F.I. adrenaline/F.I. noradrenaline (at higher wavelength)}}{\text{F.I. adrenaline/F.I. noradrenaline (at lower wavelength)}}$$

F.I.=Fluorescence intensity

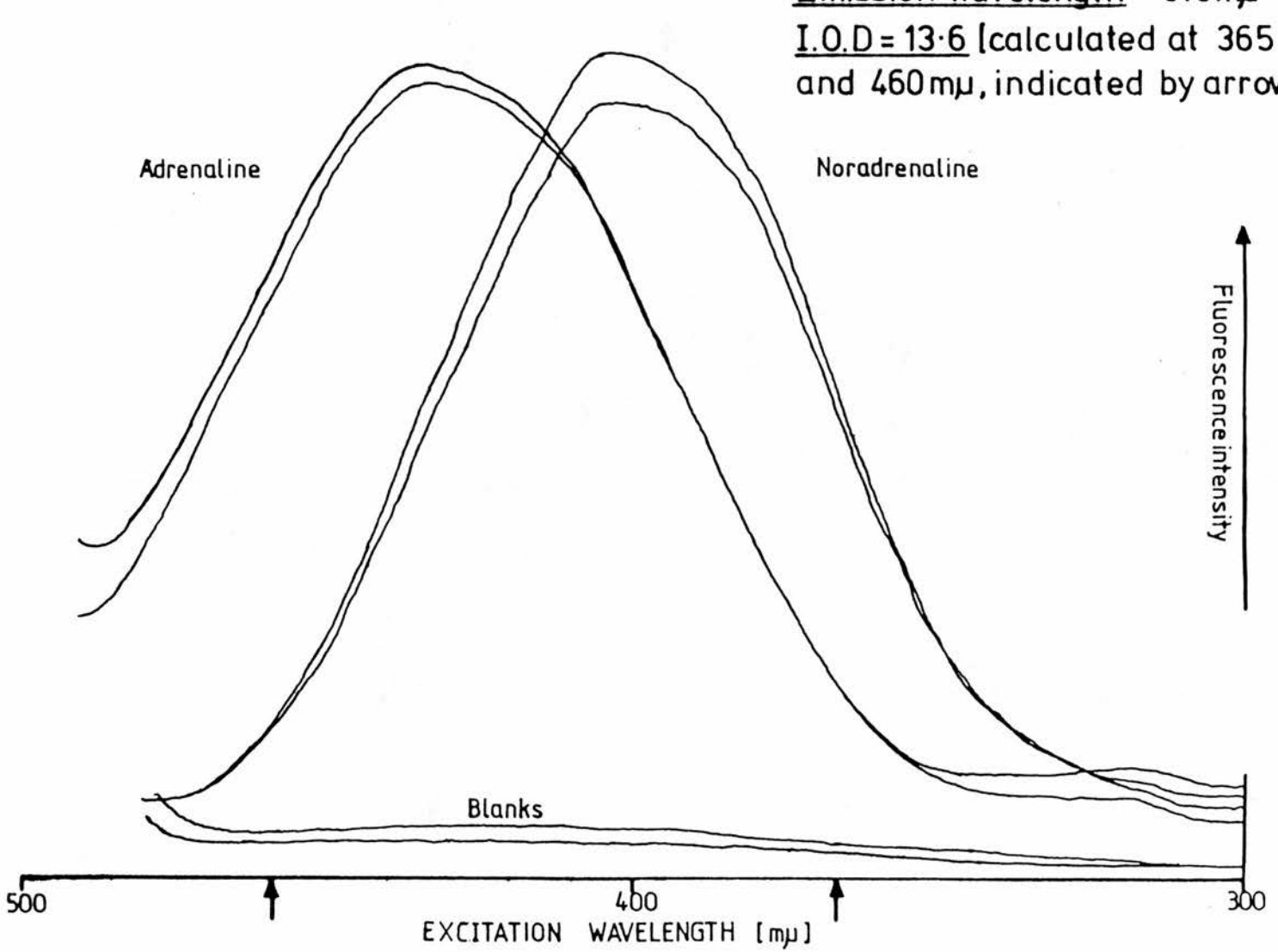
510mμ is the emission wavelength at which the I.O.D. is high and the fluorescence of adrenaline and noradrenaline relatively equivalent (Ellis, 1983).

The excitation wavelengths that gave the highest I.O.D. were calculated for each assay. An I.O.D. of greater than 9 was considered acceptable. Figure M2 shows a typical chromatogram of 100ng adrenaline and noradrenaline, indicating the excitation wavelength used for calculation of I.O.D. and catecholamine values. The excitation wavelengths used usually fell between 350 to 370 (lower), and 450 to 470 (higher).

The mean values of each sample and blank duplicate were used, and the value of the blanks at the lower and higher wavelengths subracted from those of the catecholamines before calculating their values.

Figure M2

Reduced photocopy of a typical fluorescence spectra of 100ng of adrenaline and noradrenaline.



4.3. Calculation of adrenaline and noradrenaline

The relationship between the fluorescence intensity and the concentration of catecholamine is assumed to be linear. The fluorescence of adrenaline and noradrenaline are also assumed to be additive.

So, for any given point on the specrum-:

$$F = yN + xA$$

Where F = Fluorescence intensity, A and N are the fluorescence intensities per nanogram of adrenaline and noradrenaline and y and x are the unknown amounts of noradrenaline and adrenaline in the sample.

If the fluorescence readings are teken at two wavelengths, one high (H) and one low (L), then a pair of simultaneous equations can be written (Bertler, Carlsson and Rosengren, 1958)-:

$$F(L) = yN(L) + xA(L) \text{ and } F(H) = yN(H) + xA(H)$$

Solving these equations gives -:

$$y = \frac{F(L) \times (A(H)/A(L)) - F(H)}{N(L) \times (A(H)/A(L)) - N(H)} \quad \text{and} \quad x = \frac{F(H) - yN(H)}{A(H)}$$

A computer program was used to calculate the unknown amounts of

noradrenaline and adrenaline for each sample. After calculation, the percentage recovery for each batch of samples was calculated. The maximum fluorescence (MFI) intensity of adrenaline and noradrenaline column standards was taken as a percentage of the MFI of adrenaline and noradrenaline non-column standards. Each calculated value was adjusted accordingly. The percentage recoveries for ten assays was 95 ± 2.1%.

In order to satisfy myself that these procedures gave accurate values to unknown adrenaline and adrenaline mixtures, I extracted and assayed a series of known mixtures, calculated their values and compared the calculated value to the actual value in the mixture. The results are shown below.

Amount added (ng)		Amount calculated (ng)	
Adrenaline	Noradrenaline	Adrenaline	Noradrenaline
100	100	98.4	97.3
100	100	98.1	98.5
80	20	80.9	20.1
80	20	79.2	19.5
60	40	61.3	37.2
60	40	60.8	37.7
50	50	49.8	50.0
50	50	49.6	49.2

I repeated this series of determinations at fortnightly intervals to ensure the assay was giving accurate results.

Preparation of Amberlite CG50 columns

Introduction

Amberlite CG50 was the resin of choice, as a 5cm column was found to give recoveries consistently over 90%. In addition, with the stronger sulphonic acid resins such as CG120, up to 4ml of 1N HCl is required to elute the catecholamines and it is necessary to neutralise each eluate to pH 6.5 before the THI reaction can be induced. The catecholamines can be eluted from the CG50 resin, which is a weak carboxylic acid resin, using 1ml of 2/3M Boric Acid, previously adjusted to pH 6.5. 1ml of eluate is also the ideal volume for the CSCA assay.

Preparation of the resin

Recycling the resin between the contracted hydrogen form and the expanded sodium form expels impurities and greatly improved the efficiency of the resin. I repeated the following preparation at fortnightly intervals, and each batch of resin was kept no longer than two months.

The following preparation has been adapted from that first described by Renzini, Brunori and Valori (1970).

1. The resin was soaked overnight in 2N HCl.(The same volume of HCl as resin was used.)
2. The HCl was removed and the resin washed several times with

distilled water. The water was renewed between washes. This removed the fines contained in the resin.

3. The resin was thoroughly shaken in 2N NaOH (same volume as resin). The NaOH was removed.

4. The resin was washed in distilled water until a neutral pH was obtained.

5. The resin was thoroughly shaken in 2N HCl.

6. 4. was repeated.

7. 3.- 6. were repeated three times.

8. The resin was thoroughly shaken in 0.4M disodium phoshate buffer (pH 6.5) three times.

9. The resin was stored at 2-4°C.

Preparation of columns

A glass wool plug was placed at the end of each column (0.25 cm i.d.) to retain the resin. The resin was loaded up to 5cm into the columns, making sure that no air bubbles accumulated in the resin. The columns were loaded immediately before use. Before the samples were added, 10 mls of 0.4M disodium phosphate buffer (pH 6.5) was run through the columns. The pH of the eluant was checked, and if not at 6.5, more buffer was run through until the pH was 6.5. This is the optimum pH for the positively charged catecholamines to be retained by the resin. The columns were then ready for use.

High performance liquid chromatography with electrochemical detection for the assay of catecholamines

Advantages of High Performance Liquid Chromatography (HPLC) over CSCA

In order for CSCA to remain a satisfactory, consistent assay technique it was necessary to ensure that each reagent added to induce the THI reaction was added at a defined and accurately controlled time point. I was becoming increasingly frustrated by the hydraulics and dispensing pipettes of the Unicam AC60 autoanalyser becoming jammed. This frequently occurred in the middle of a throughput of 24 samples and jeopardised the successful assay of all samples still to pass under the dispensors. The volume of each reagent was also becoming increasingly more difficult to control due to the dispensing pipettes often failing to dispense their total volume. These faults were undoubtedly due to the age of the autoanalyser and its failing hydraulics.

I had become aware of the simplicity of HPLC as a means of analysing catecholamines and on investigating further discovered that it had many advantages over CSCA-:

1. The catecholamines are extracted in alumina. This procedure is much simpler and less time consuming than that previously described using Amberlite CG50 resin.
2. Catecholamines are eluted from alumina using 0.1N perchloric acid which could then be injected directly into the HPLC system. This avoids the time consuming proceedures required to induce the THI reaction.

3. HPLC separates noradrenaline and adrenaline and each is illustrated as a separate peak on the chromatogram. The height of each peak is directly proportional to the catecholamine concentration and enables a much simpler calculation of unknown catecholamine concentrations, which does not require computer analysis.
4. An internal standard is added to each plasma sample before extraction. The values of the catecholamines are calculated using the peak height of the internal standard. Each sample therefore has its own standard and, during the calculation of catecholamine values, each is automatically adjusted for any loss during the extraction procedure. With CSCA there is no internal standard. A percentage recovery of adrenaline and noradrenaline is calculated and every sample in a batch adjusted accordingly. This does not allow for any variations that may occur between individual sample.

These were the main reasons I had for converting to HPLC for the analysis of catecholamines. Catecholamines were estimated by HPLC in dog 12 and all subsequent dogs.

An introduction to HPLC with elecro-chemical detection

The first report of the application of HPLC with electro-chemical detection (HPLC-ElCD) to the analysis of catecholamines was made by Kissinger, Ref Shauge, Dreiling and Adams (1973), and its subsequent use for the determination of plasma catecholamines was described by Hallman, Farnebo, Hamberger and Jonsson (1978).

Several modes of HPLC have been used: ion-exchange, reverse-phase

and reverse phase with ion-pairing (RP-IP) chromatography. I shall breifly discuss the ion-exchange and reverse phase modes before discussing RP-IP in more detail.

Cation-exchange chromatography

This mode of chromatography depends on the catecholamines being retained by an ion-exchange resin and was the method used by Kissinger etal (1973) and Hallman etal (1978). They used a relatively large and irregularly shaped strong cation-exchange material but the chromatograms obtained often showed broad peaks and gave a poor separation of noradrenaline from the solvent front. This was overcome by Hjemdahl (1984) and Allmark and Hedman (1979) who used a microparticulate cation-exchange material. However, the ion-exchange packings still have several disadvantages: They are less efficient, less reproducible and less stable than reverse phase columns and the number of packings available is limited.

Reverse-phase chromatography

The potential of this technique for catecholamine analysis was first demonstrated by Molnar and Horvath in 1978. Reverse-phase chromatography is so named because the column particles (stationary phase) are coated with carbon chains, usually 18 moieties in length. They provide efficient separation of compounds with varying lipophilicity and this mode of chromatography is very versitile in that the separation of compounds can be affected in many ways. Varying the pH of the mobile phase (affecting ionization) in catecholamine

analysis influences their retention. Although this mode of chromatography is very efficient, it has certain disadvantages in comparison with RP-IP chromatography. Many interfering substances tend to be retained along with the catecholamines, and interfere with the chromatogram, and there is often a poor separation of noradrenaline from the solvent front.

Reverse-phase-Ion pairing chromatography

The development of this mode of chromatography has greatly improved the sensitivity, efficiency and versitility of HPLC for catecholamine analysis (Moyer and Jiang, 1978). In RP-IP, ion-pairing reagents are added to the mobile phase to increase retention, and therefore separation, of charged solutes. The retention is believed to occur through ion-pair formation in the mobile phase (Terweij-Groen, Heemstra and Kraak,1979).

The main equilibria involved are as follows-:

1a. Ion-pair formation in the mobile phase with the anion-pairing reagent, L^-

$$CA^+ (m) + L^- (m) \leftrightarrow (CA^+ L^-)(m)$$

1b. Reversible binding of the ion-pair to the hydrocarbonaceous surface-:

$$(CA^+ L^-)(m) \leftrightarrow (CA^+ L^-)(s)$$

CA = catecholamine

m and s = mobile and stationary phase respectively.

2. Dynamic ion exchange, the solute molecule forming a complex with the ligand already adsorbed-:

$$CA^+ (m) + L^- (s) \leftrightarrow (CA^+ L^-)(s)$$

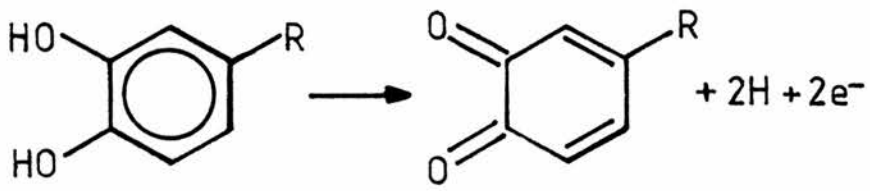
3. Dynamic complex exchange between the ion-pair formed in the mobile phase and the ion-pairing reagent bound to the column-:

$$(CA^+ L^-)(m) + L^- (s) \leftrightarrow (CA^+ L^-)(s) + L^- (m)$$

The ion-pairing reagent most frequently used is sodium octane-1-sulphonate which gives an excellent selectivity with a minimum equilibration time. Addition of methanol and citrate ions in the mobile phase also affects retention. Citrate ions in the mobile phase lower the retention of catecholamines by exerting an electrostatic attraction on the "pairs" (Krstulovic, 1982).

Electrochemical detection

Electrochemical detection relies on catecholamines being oxidised to the corresponding orthoquinones at the surface of a graphite electrode-:



Catecholamine Orthoquinone

The anodic current produced is directly proportional to the number of solute molecules in contact with the electrode (Kissinger, Bruntlett and Shoup, 1981). This anodic current can be converted into a voltage which drives a pen recorder to give the relevant chromatogram.

Extraction of catecholamines

Introduction

Catecholamines undergo complex binding to aluminium oxide (alumina). Previously alumina was "activated" as described by Anton and Sayre (1962). Present preparations of alumina no longer require activation. This method of extraction was chosen in favour to that previously used for CSCA for the following reasons-:

1. The alumina does not require the time consuming preparation the Amberlite CG50 resin requires.
2. Alumina extraction can be carried out in test-tubes. This avoids the need to prepare and pack columns as with the Amberlite CG50 method.
3. As only 20µl of eluate is injected into the HPLC system, the catecholamines need to be eluted in a very small volume of eluate. 1ml of boric acid is required to elute the catecholamines from the Amberlite CG50 resin column. 200µl of 0.1M perchloric acid will elute the catecholamines from the alumina, increasing the concentration of catecholamines injected, decreasing the need for a very high sensitivity.

The extraction procedure I adopted is a modification of those described by Hjemdahl, Daleskog and Kahan (1979) and Davies and Molyneuk (1982).

Extraction procedure

The adrenal venous blood samples were collected and stored as described for CSCA. The reagents used were-:

1. 0.1M perchloric acid (PCA).
2. Tris buffer, pH 8.6 (12g Tris, 2g EDTA, 100ml distilled water, pH corrected with HCl).
3. Internal standard (100ng per 200µl of dihydroxybenzylamine (DHBA) in 0.1M PCA).
4. Alumina (Sigma, chromatographic, Acid Type WA-1). Stock solutions of 500ngml^{-1} DHBA were kept refrigerated for up to one month. All standards were made up in 0.1M PCA.

The following protocol was followed-:

1. 20-30mg of alumina was placed in conical bottomed test-tubes.
2. 2ml of sample plus 200µl of the DHBA internal was added to the alumina. (This gives an internal standard chromatogram peak of 10ng DHBA.)
3. The pH was adjusted to 7.8-8.2 with Tris buffer.
4. The samples were mixed on a "Spiromix" blood mixer for 20 minutes.
5. The alumina was allowed to settle and the supernatant removed.
6. 1- 2ml ice-cold distilled water was added and mixed.
7. 5. and 6. were repeated to give three washes in the distilled water.

8. The alumina was allowed to settle and as much of the supernatant was removed as possible.

9. 200µl 0.1M PCA was added to the alumina.

10. The tubes were mixed in a Vortex shaker for 20 minutes.

11. The alumina was allowed to settle and the 0.1M PCA eluates dispensed into clean test-tubes.

12. 20µl of eluate was injected into the HPLC system.

PCA both extracts and prevents oxidation of the catecholamines.

The HPLC-ElCD system

A schematic representation of the system used is illustrated in figure M3.

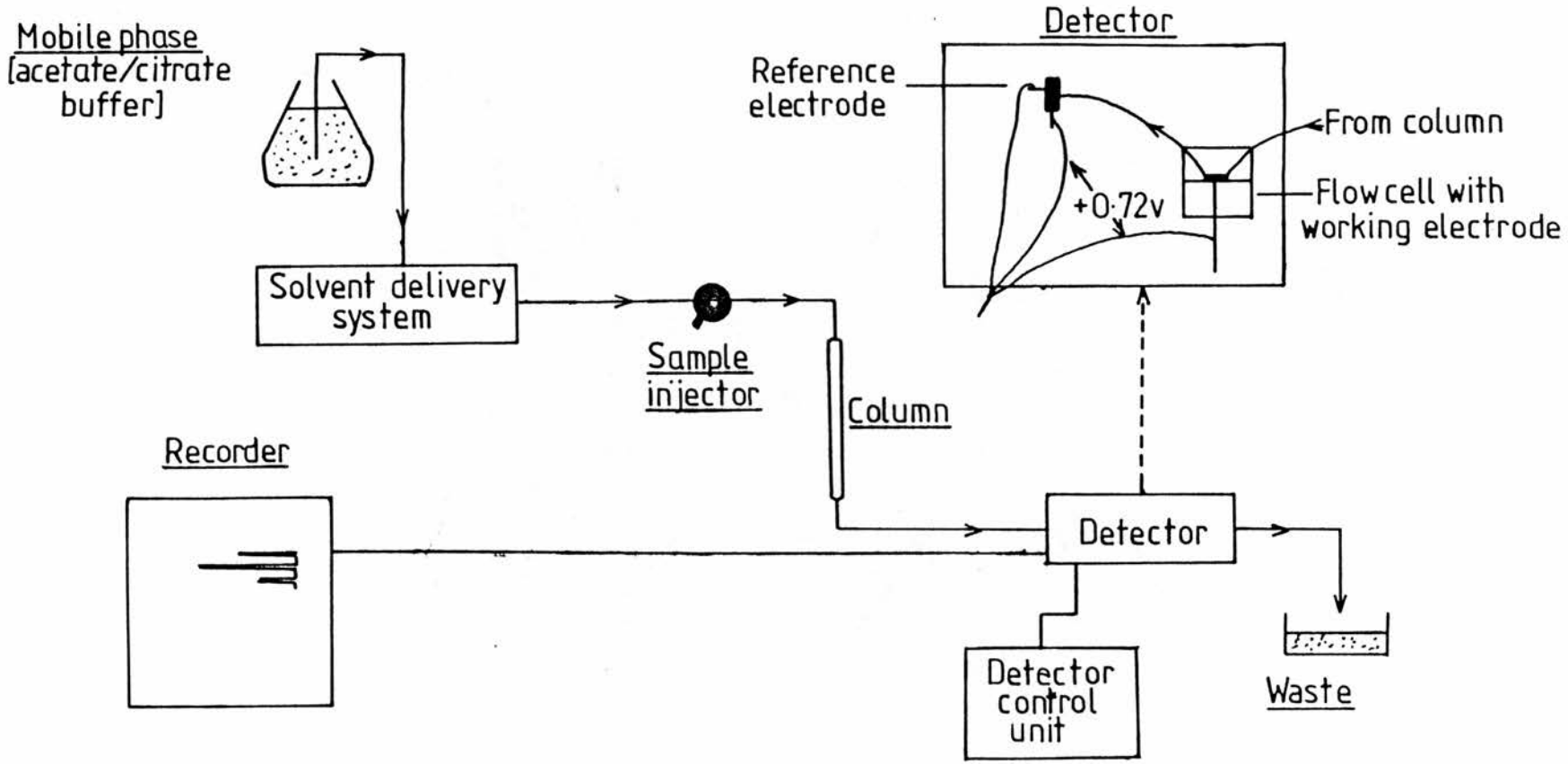
The mobile phase used was an 0.1M acetate/citrate buffer, pH 5.2 (11.5g citric acid, 13.6g sodium acetate, 4.8g NaOH, 0.74g EDTA, 0.2g sodium octane-1-sulphonate, in 2L of distilled water/10% methanol, the pH was adjusted with glacial acetic acid). The buffer was filtered and degassed before use, to minimise the occurrence of bubbles forming on the glassy carbon electrode. This was done by by filtering the buffer through a millipore filter (0.22um) into a vacuum flask which was evacuated using a water pump. A fresh buffer was made up every month.

The liquid chromatograph comprised of the following-:

1. Solvent delivery system - Gilson 303 pump plus a Gilson 802

Figure M3

A schematic representation of the High performance Liquid Chromatography system used for the analysis of catecholamines.



manometric module. Solvent delivered at 1mlmin^{-1}.

2. Sample injector - Rheodyne Model 7125 injection valve fitted with a 20µl sample loop.

3. Precolumn - 1.5mm ID, 20mm length, packed with Partisil - 10 - SCX, Strong Cation Exchanger (Whatman).

4. Column - "Ultrasphere-ODS" 4.6mm ID, 25cm length, packed with 5µ diameter ultrasphere IP particles.

5. Detector system - B.A.S. LC-4B Ampometric Detector plus an electrochemical detector fitted with a TL-5 cube, glassy carbon cell. The oxidation potential applied was +0.72 volts.

6. Servoscribe flat-bed recorder.

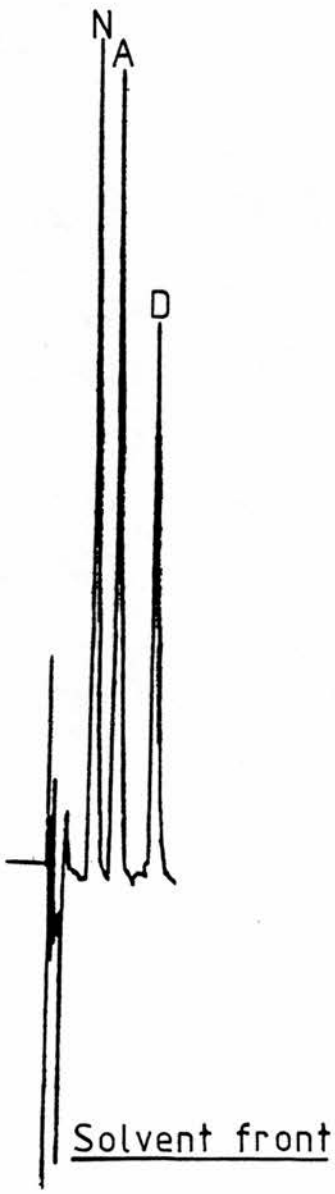
Before any samples were injected into the HPLC system, the electrode was "conditioned". This prevents any drift and noise. The electrode was switched off, the voltage switched to +1.5 volts, the offset switched off and the attenuation set at 500nAmp. The buffer flow rate was set at 0.3mlmin^{-1}, then the electrode switched on and left for at least one hour. The electrode was then switched off, the voltage switched to +0.72 volts, the offset to on and the attenuation to the required setting (usually 5). The buffer flow rate was set at 1mlmin^{-1} and the electrode switched on. After a steady baseline was obtained (10-30 minutes), the samples were injected.

An example of a typical chromatogram is shown in figure M4. It was possible to inject samples between the adrenaline and DHBA peaks of the prece ding chromatogram without the solvent front interfering with the DHBA peak. The approximate retention time for noradrenaline was 2 minutes, for adrenaline was 4 minutes and for DHBA was 6.5 minutes.

Figure M4

H.P.L.C. TRACE OF ADRENALINE [A], NORADRENALINE [N] AND DIHYDROXYBENZYLAMINE [D] STANDARDS [10ng].

Applied e.m.f. = +0·72 volts
Range = 10
Offset = 5
Flow = 1mlmin^{-1}
Chart speed = 2mmmin^{-1}



Calculation of catecholamine values

The value of adrenaline and noradrenaline was calculated using the following equation-:

$$x = \frac{\text{Level of DHBA} \times \text{Peak height of X}}{\dfrac{\text{Peak height (CA)}}{\dfrac{\text{Ratio Peak height (CA)}}{\text{Peak height (DHBA)}}}}$$

X = Unknown value of the catecholamine

(CA) = Peak height of catecholamine standard

(DHBA) = Peak height of DHBA standard

The ratio was calculated at monthly intervals by compiling a graph for varying concentrations of catecholamines and DHBA vs their peak heights and calculating the ratio (see figure M5).

Table M1 shows a comparison of the catecholamine values obtained by fluorimetry and HPLC in adrenal blood samples. The major difference between the two was the percentage of noradrenaline present in each sample. The total catecholamine values can be seen to agree very well. I suspect that the differences in the percentage of noradrenaline lay in the age of the autoanalyser and inconsistency in the volumes of reagents delivered to each sample, due to the failing hydraulics in the dispensing system.

Figure M5

Graph showing concentration of noradrenaline [NA], adrenaline [A] and dihydroxybenzylamine [DHBA] vs peak height [PH] of a High Performance Liquid Chromatogram.

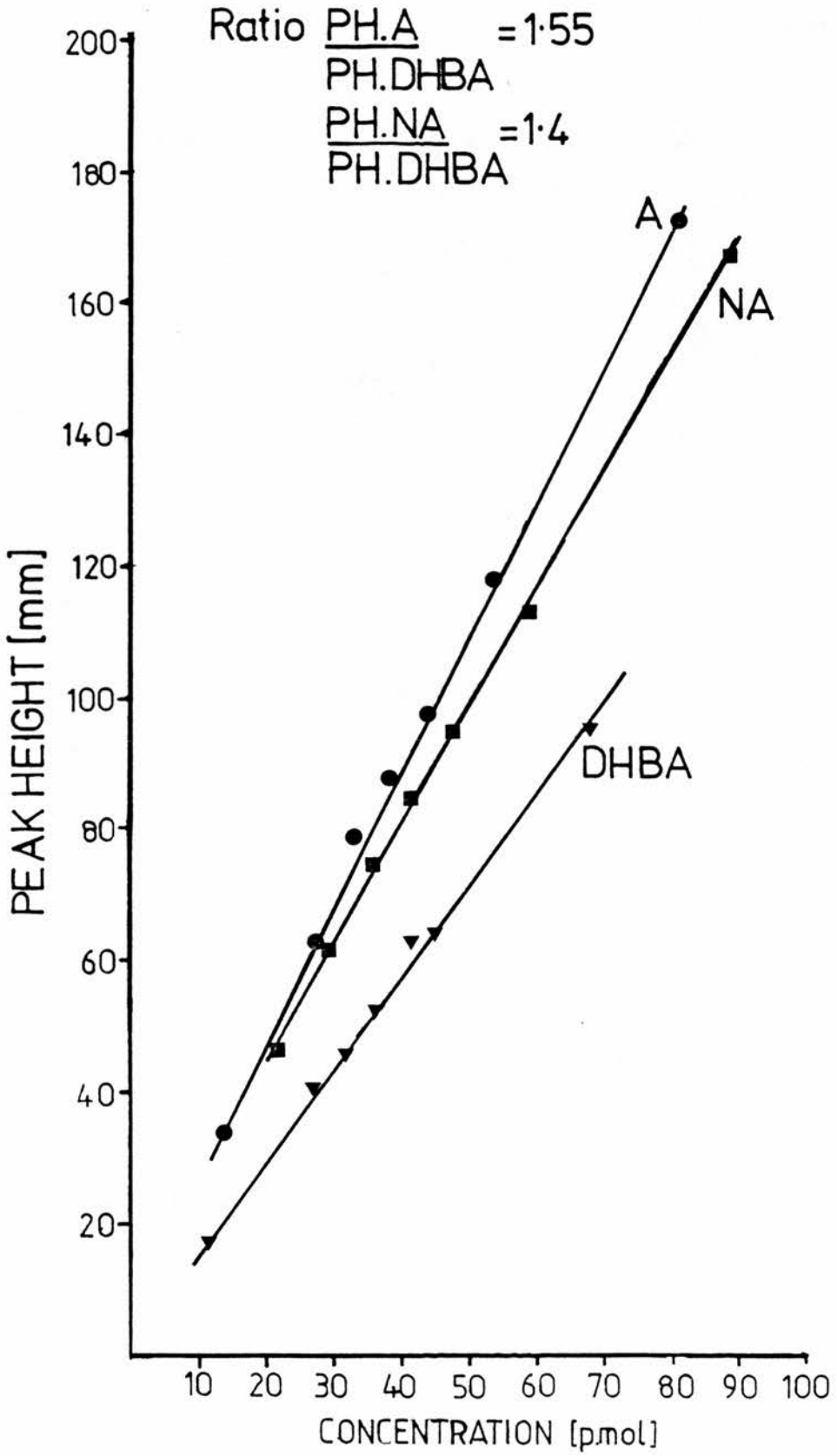


Table M1

Comparison of catecholamine estimations by Fluorimetry and High performance Liquid Chromatography (HPLC).

Sample	Values obtained by fluorimetry (ng)				Values obtained by HPLC (ng)			
	NA	A	NA+A	%NA	NA	A	NA+A	%NA
1	57	190	247	23	50	205	255	20
2	190	435	625	30	133	476	609	22
3	31	66	97	32	18	77	95	20
4	42	75	117	36	25	109	134	19
5	25	209	234	11	58	229	287	20
6	20	154	174	11	44	168	212	26
7	116	230	346	34	62	234	296	21
8	78	209	287	27	57	232	289	20
9	191	331	522	37	119	422	541	22
10	201	389	590	34	124	475	599	21
11	361	632	993	36	183	767	950	19
12	263	633	896	29	150	633	783	19
Total	1573	3553	5126		1023	4027	5050	
Mean ± SE				28.3 ± 2.6				20.8 ± 0.6

NA = Noradrenaline A = Adrenaline

Whole animal experiments

Anaesthesia

Dogs and cats were anaesthetised with sodium pentobarbitone (30mgkg^{-1} iv.). Anaesthesia was maintained by an additional iv. injection if a corneal reflex became evident during the experiment. Pentobarbitone was the anaesthetic of choice as work previously carried out in this laboratory has shown that the adrenal medulla is more responsive to baroreceptor stimulation under pentobarbitone, than the other anaesthetic of choice, chloralose (Critchley, Ellis and Ungar, 1980).

Respiration, acid-base balance and temperature control

The trachea was cannulated and connected to a Starling "Ideal" pump. The lungs were ventilated with a metered oxygen-nitrogen mixture. Regular arterial blood samples were obtained and $PaCO_2$,PaO_2 and pH measured on a Radiometer BMS 3 analyser. The oxygen-nitrogen mixture was adjusted to maintain $PaCO_2$ at 5kPa (39mmHg) in dogs, and 4kPa (30mmHg) in cats and PaO_2 above 20kPa (150mmHg). Any base deficit was corrected, to maintain pH at 7.4, by injecting an appropriate volume of 1M sodium bicarbonate, according to the Singer-Hastings nomogram (Singer and Hastings, 1948). Respiration rate was fixed at 25 per minute. The stroke volume was adjusted, if required, to maintain blood gas levels. Body temperature was held at 37°C by a heating lamp and pad, linked to a rectal thermistor probe.

Maintainance of systemic arterial blood pressure

Blood pressure was monitored throughout each experiment and, if necessary, maintained by iv. administration of dextran (40% in saline prewarmed to 37°C in a water bath). Blood pressure falls as a result of blood loss during surgery, handling of viscera and prostaglandin release by surgical trauma (Terragno, 1977). Bladder reflexes resulting from a distended bladder also induce a fall in blood pressure (Taylor, 1968) and the bladder was often drained using a catheter or suprapubic puncture to avoid this. It was usually not necessary to administer dextran prior to captopril administration. Dextran was administered by a continuous iv. infusion following captopril due to the hypotensive action of captopril, in order to prolong survival of the anaesthetised preparation.

Bilateral carotid bifurcation perfusion

Surgery

Both carotid arteries were exposed. The superior thyroid arteries were immediately tied off, and loose ligatures placed around the common carotid arteries, the external carotid arteries (cranial to the origin of the lingual arteries), the lingual arteries and the vagi. Care was taken to dissect well away from the carotid sinuses which are located at the carotid bifurcations, located at the point where the naso-pharangeal nerve crosses the carotid artery. The external carotid arteries were located by following the lingual arteries down to the carotid arteries.

Cannulation and perfusion

A diagram of the perfusion system for the carotid bifurcations and collection of adrenal venous blood samples is shown in figure M6.

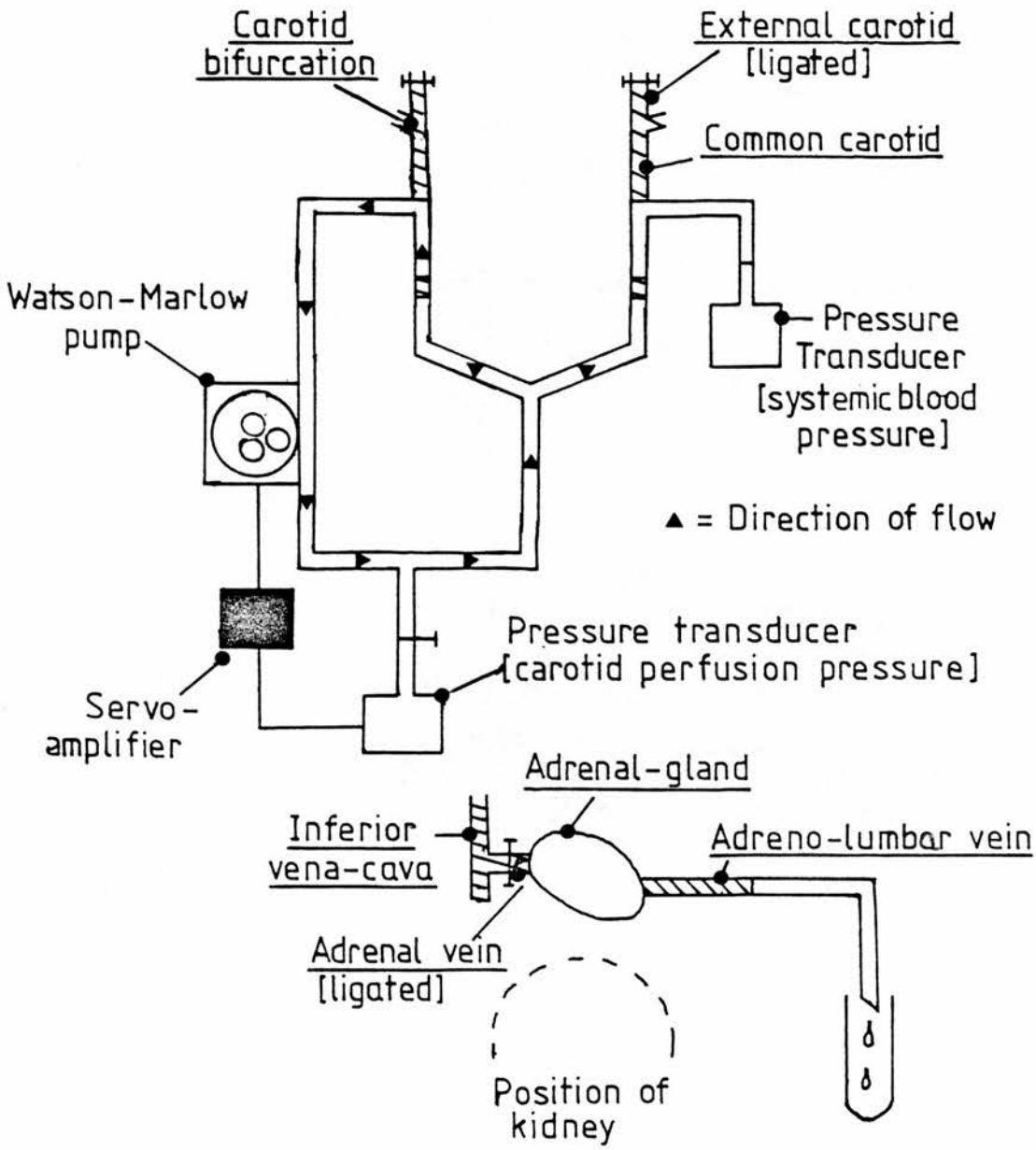
Both common carotids were cannulated both ways and blood from one (usually the right) was perfused into both, towards the head, using a Watson- Marlow perfusion pump. The left carotid artery was cannulated to monitor systemic blood pressure. The external carotid arteries were tied off. The lingual arteries were not ligated in order to ensure an adequate blood flow through the carotid bifurcations. Both vagi were cut to abolish secondary reflexes from the thoracic receptors.

A pressure transducer was connected to the perfusion circuit to record carotid perfusion pressure (CPP), and was linked via a servo amplifier to the Watson-Marlow perfusion pump. The servo amplifier exerts a negative feedback to control the speed of the perfusion pump, to maintain a constant CPP, set by a "clock" potentiometer. The clock potentiometer is calibrated in mmHg and the CPP could be varied by adjusting the potentiometer clock.

Baroreceptor stimulation

Baroreceptor stimulation, or baroreceptor "tests" were performed by lowering the CPP by 40mmHg for the required length of time, usually ten minutes. A reflex increase of systemic blood pressure, following baroreceptor stimulation, was evidence that baroreceptor

Figure M6 Diagram of perfusion of the carotid bifurcations and collection of adrenal venous blood.



stimulation was successful. Figure M7 shows a typical trace obtained during baroreceptor stimulation, and illustrates the reflex increase in systemic blood pressure which accompanies each successful baroreceptor test.

Collection of adrenal venous blood samples

In the dog the venous effluent from the adrenal gland drains into the adrenolumbar vein. This runs a short distance before fusing with the inferior vena cava. In the cat it may fuse with either the inferior vena cava or the renal vein. This distance of vessel, referred to as the adrenal vein, is too short to cannulate and so the adrenolumbar vein is cannulated in a retrograde fashion to allow collection of adrenal venous blood. Figure M6 shows a diagram of the cannulation.

The abdomen was opened and the adrenal gland located. The adrenal vein was isolated, and a loose ligature placed around it. The adrenolumbar vein was isolated, and two loose ligatures placed around it. All small branches of the adrenolumbar vein, between the point of cannulation and the gland, were tied off.

A soft, flexible polythene tubing, selected to fit the adrenolumbar vein was used for cannulation. The adrenolumbar vein was cannulated and the cannula pushed past the adrenal gland and positioned so that its tip lay at the point where the vein leaves the gland. Prior to tying off, the adrenal vein was occluded by pulling on the loose ligature. If this resulted in a stoppage of adrenal blood

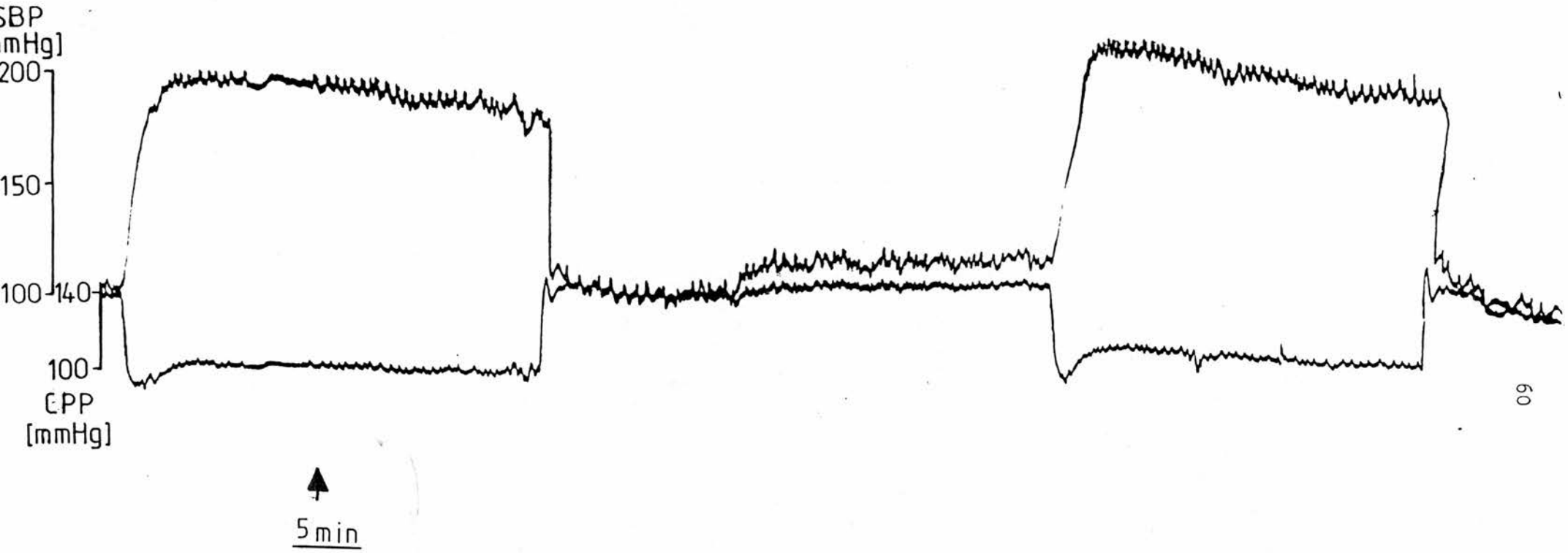


Figure M7

PHOTOCOPY OF TRACE FROM DOG 7 SHOWING THE REFLEX INCREASE IN SYSTEMIC BLOOD PRESSURE [SBP] FOLLOWING A LOWERING IN CAROTID PERFUSION PRESSURE [CPP].

flow through the cannula, this indicated that the cannula had been pushed too far and was pulled back until flow resumed. The cannula was then secured, and the adrenal vein tied off. A small hole was punctured through the abdomen wall and the cannula pulled through.

The adrenal venous blood was collected in cooled, graduated centrifuge tubes. The left saphenous vein was also cannulated, and any adrenal blood not collected was immediately returned to the animal via the saphenous vein, using a Watson-Marlow pump. Any additional anaesthetic, drugs and dextran were also administered into the saphenous vein.

Splanchnic nerve stimulation

Figure M8 shows the preparation of the left adrenal gland for stimulation of the splanchnic nerve and collection of adrenal venous blood.

The main splanchnic nerve trunk was located and a length isolated. Two loose ligatures were placed around the nerve. Any smaller branches of the nerve were isolated, tied off and severed, and a region around the nerve was thoroughly crushed, using Spencer-Wells forceps, to ensure maximum denervation of the adrenal medulla. The main trunk was then cut some 1-1.5 inches from the gland, and a bipolar stimulating electrode hooked under the nerve, towards the pripheral cut end. The two loose ligatures were used to raise the nerve over the electrode. The nerve was stimulated using a Tektronix stimulator.

Preparation of the left adrenal gland for stimulation of the splanchnic nerve and collection of adrenal venous blood.

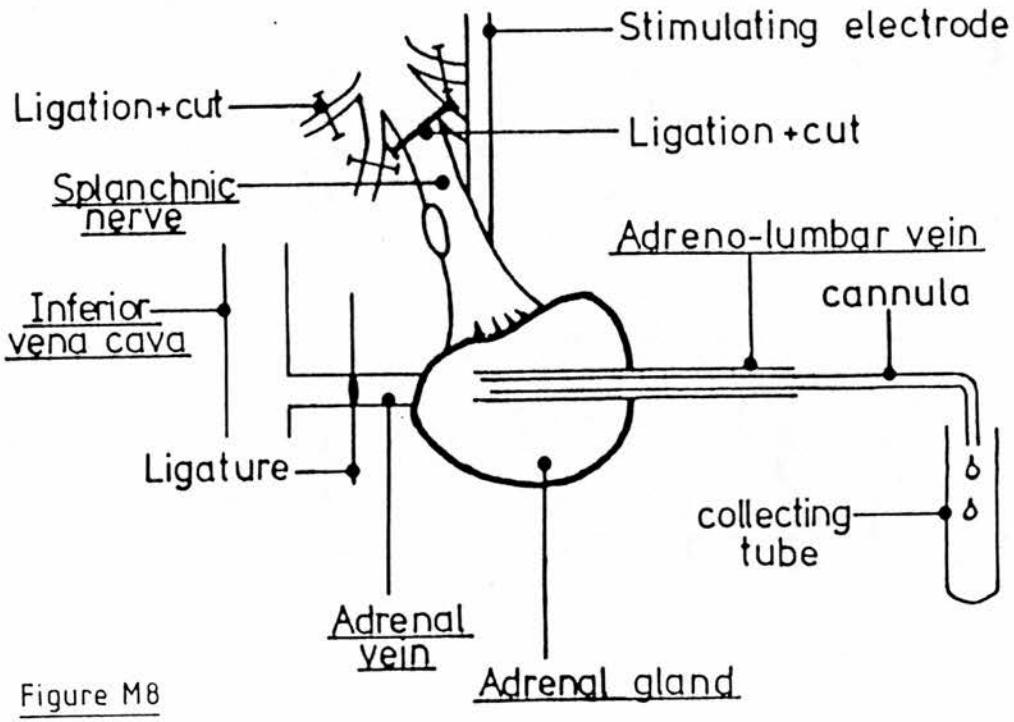


Figure M8

Summary of the experimental protocol for perfusion of carotid bifurcations and collection of adrenal venous blood samples

1. Surgery was performed to:-

a. Cannulate the left saphenous vein

b. Prepare the carotid bifurcations for perfusion

c. Prepare the left adrenal gland for collection of adrenal venous blood.

2. Half an hour was allowed for haemostasis.

3. 500 $i.u.kg^{-1}$ heparin was administered.

4. The animal was put onto the respiratory pump.

5. The carotid bifurcations were cannulated and perfused. Carotid perfusion pressure and systemic blood pressure were recorded.

6. Both vagi were cut.

7. The adrenolumbar vein was cannulated, the adrenal vein tied off and adrenal blood collected.

8. Perfusion of excess adrenal blood into the saphenous vein was started.

9. Blood gases and acid-base balance were adjusted if necessary (this was repeated frequently throughout each experiment).

10. The preparation was allowed up to 30 minutes to settle before baroreceptor tests were performed.

Summary of the experimental protocol for stimulation of the splanchnic nerve and collection of adrenal venous blood samples

1. Surgery was performed to:-

a. Cannulate the left saphenous vein.

b. Prepare the left common carotid for cannulation (to record systemic blood pressure).

c. Prepare the left adrenal gland for adrenal venous blood collection and splanchnic nerve stimulation.

2. Half an hour was allowed for haemostasis.

3. 500 $i.u.kg^{-1}$ heparin was administered.

4. The animal was put onto the respiratory pump.

5. The left common carotid was cannulated and systemic blood pressure recorded.

6. The left adrenolumbar vein was cannulated, the adrenal vein was tied off and adrenal venous blood collected.

7. The bipolar electrode was positioned under the main splanchnic nerve trunk.

8. Perfusion of excess adrenal blood into the saphenous vein was started.

9. Blood gases and acid-base balance were adjusted if necessary.

10. The preparation was allowed up to 30 minutes to settle before splanchnic nerve stimulation was commenced.

Details of the experimental protocol adopted during baroreceptor stimulation experiments

Unless otherwise stated in the "Results" section, the following experimental procedure was adopted:-

1. A control 1 minute adrenal blood sample was collected.

2. Baroreceptor stimulation was started and a stop-clock started simultaneously.

3. 1 minute adrenal venous blood samples were collected during baroreceptor stimulation from 1-2, 5-6 and 9-10 minutes.

4. Baroreceptor stimulation was stopped and the preparation allowed 20 minutes to recover.

5. Steps 1-4 were repeated.

6. Drug A was administered. A list of the drugs used, the means of administration and the time allowed for them to take effect is listed later.

7. Steps 1-5 were repeated.

8. Drug B was administered.

9. Steps 1-5 were repeated.

10. Drug C was administered.

11. Steps 1-5 were repeated.

Where plasma renin activity was measured, 2ml carotid arterial blood samples were collected during each adrenal blood collection period. The samples were centrifuged as for adrenal blood samples and the plasma removed and frozen. Plasma renin activity was measured by radioimmunoassay (RIA).

Where adrenocorticosteroid output was measured (by RIA), additional 1 minute adrenal blood samples were collected each time step 1 was carried out.

I shall outline the main drugs used in parts 1-5 of the "Results" section. Any additional drugs or procedures used will be indicated in the "results" section.

Drugs used

Part one

Drug A - Captopril, 25 mg iv., allowed 20 minutes to take effect.

Drug B - Angiotensin II - dosage indicated in the "Results" section, continuous infusion, allowed 20 minutes to take effect.

Part two

1. Drug A - Captopril.
   Drug B - Angiotensin II.
   Drug C - Cycloheximide, 50 $mgkg^{-1}$ iv., allowed 15 minutes to take effect.

2. Drug A - Captopril.
   Drug B - Adrenocorticotrophic hormone (ACTH), 100µg iv., allowed 20 minutes to to take effect.

3. Drug A - Cycloheximide.
   Drug B - Captopril.
   Drug C - Angiotensin II.

Part four

Drug A - Naloxone, $0.3mgkg^{-1}$, continuous iv. infusion, $1mlmin^{-1}$, allowed 15 minutes to take effect.

Drug B - Captopril.

Drug C - Naloxone.

Part five

Drug A - Indomethacin, 5mgkg^{-1} iv., allowed 15 minutes to take effect.

Drug B - Captopril.

Details of the experimental protocol adopted during splanchnic nerve stimulation experiments

Unless otherwise stated in the "Results" section, the following experimental protocol was followed-:

1. A control 1 minute adrenal blood sample was collected.
2. Splanchnic nerve stimulation (10 volts, 2ms, 10 pulses per second) was started and a stop-clock started simultaneously.
3. A 1 minute adrenal venous blood sample was collected 30 seconds after onset of stimulation.
4. The stimulation was stopped and the preparation allowed 20 minutes to recover.
5. Steps 1-4 were repeated.
6. Drug A was administered (see later).
7. Steps 1-5 were repeated.
8. Drug B was administered.
9. Steps 1-5 were repeated.
10. Drug C was administered.
11. Steps 1-5 were repeated.

In the initial series of experiments, steps 1-4 were repeated for four frequencies of stimulation - 2.5, 5, 10 and 20 pulses per second and step 5 was omitted.

I shall outline the main drugs used in parts 3 - 5 of the "Results" section. Any additional drugs or proceedures used will be indicated in the relevent "Results" section. The same dosages, means of administration and time allowed to take effect described previously were used.

Part 3

1. Drug A - Captopril.
   Drug B - Angiotensin II.

2. Drug A - Saralasin 10$\mu gmin^{-1}$ kg iv., allowed 10 minutes to take effect.

Part four

Drug A - Naloxone.
Drug B - Captopril.
Drug C - Naloxone.

Part five

Drug A - Indomethacin.
Drug B - Captopril.

Measurement of reflex changes in the resistance of the vascular bed of the hind limb, in the anaesthetised dog.

Dogs were anaesthetised with sodium pentobarbitone, and surgery performed to prepare the carotid arteries for bilateral carotid bifurcation perfusion following the experimental procedure already described.

The femoral arteries in both hind limbs were located and dissected free, and loose ligatures placed around them. The preparation was allowed 30 minutes for haemostasis before administering heparin (500 i.u. kg^{-1}). The carotid arteries were cannulated for bilateral carotid bifurcation perfusion.

The femoral artery of the left limb was cannulated towards the heart, and blood from this was perfused using a Watson-Marlow pump, into the right limb, which was cannulated away from the heart. A pressure transducer was connected to the perfusion circuit to monitor hind limb perfusion pressure (HLPP). The HLPP was adjusted so that the it was approximately equal to systemic blood pressure. As flow through the perfused limb is constant, the HLPP can be taken as a measure of vascular resistance.

In order to prevent collateral blood flow between the perfused limb and the abdominal circulation, tape was tied tightly around the upper thigh. Captopril was administered into the cannulated, saphenous vein, of the non-perfused limb.

Two minute baroreceptor tests were performed before and after captopril administration, using the protocol already described, and systemic blood pressure, carotid perfusion pressure and HLPP monitored throughout the experiment.

Part 1

Increased circulating levels of AII have long been implicated in the aetiology of hypertension caused by an increase in circulating PRA (reno-vascular hypertension) (eg, Haber, 1979). The discovery of angiotensin converting enzyme (ACE) led to the development of the ACE inhibitors for the treatment of reno- vascular hypertension. AII is a known vasoconstrictor (Bohr, 1974) and it is also known to activate the sympathetic nervous system by an action on the central nervous system (Strauss, Lamdin, Smith and Bleifer, 1958; Fitzsimons, 1980). AII can be generated within the brain in connection with a brain renin-AII (R-AII) system (Severs and Daniels-Severs, 1973; Ganten and Speck, 1978) and AII, injected intracerebralventricularly (i.c.v.), can stimulate an increase in plasma catecholamines (CA) which can be inhibited by captopril (Scholkens, Jung, Rascher, Shomig and Ganten, 1980). Both plasma noradrenaline (NA) and adrenaline (A) levels increase after i.c.v. injection of AII, indicating that both the sympathoneural and sympathoadrenal axis is stimulated (Ganten, Unger, Rockhold, Schaz and Speck, 1979). AII injected i.c.v. also increases blood pressure and heart rate (Scroop and Lowe, 1969; Ferrario, Dickinson and McCubbin, 1970), an effect which can be blocked by captopril (Scholkens etal, 1980). The location of the central R-AII system correlates well with the central NA regions (Fischer-Ferrario, Nahmod, Goldstein and Finkelman, 1971). Peripherally administered AII can also exert similar effects to i.c.v. injected AII. It is thought that AII acts on the area prostrema in the floor of the 4th ventricle (Joy and Lowe, 1970a; Katic, Joy, Laverty, Lave and Scrops, 1971). This is outside the blood- brain barrier (Ferrario, Gildenburg and

McCubbin, 1972) and is associated with medullary areas, particularly the nucleus tractus solitarius which is involved in central control of blood pressure (Joy and Lowe, 1970b; Barnes, Ferrario and Conomy, 1979). So both peripherally synthesised and central AII can activate the sympathetic nervous system which leads to increased vasoconstriction, release of CAs from the adrenal medulla and also an increase in AII synthesis by sympathetic activation of renal renin release (Johnson, Davis and Witty, 1971). There is a sympathetic innervation of the juxtaglomerular apparatus (Barajas, 1964; Wagermak, Ungerstedt and Ljingquist, 1976) and renal renin release can be inhibited by blockade of adrenoceptors (Loefler, Stockigt and Ganong, 1972; Buhler, 1974).

It has been assumed that in cases of reno-vascular hypertension the effectiveness of captopril as an antihypertensive can be explained by its effectiveness in reducing both peripheral and central AII levels, causing an overall reduction in sympathetic tone. Since the introduction of captopril for the treatment of reno-vascular hypertension however, it has been discovered that captopril decreases blood pressure in man and animals wether PRA is high, normal or low (Haefely, 1972; Bengis, Coleman, Young and McCaa, 1978; Antonaccio, Rubin and Horowitz, 1980; Dollery and Miyamori, 1980). Opinions differ as to whether or not there is a significant correlation between baseline PRA and blood pressure reduction in the acute response to captopril (Andren, Karlberg, Ohman, Svensson, Asplund and Hansson, 1982). In long term treatment there appears to be no correlation between drug response and PRA, and captopril is as effective in the treatment of essential hypertension (renin independant) as

renovascular hypertension (Brunner, Gavras, Waeber, Turin and Walters, 1980; Santucci, Aguglia, De-Mattia, Ficara and Balsano, 1982; Estrada, Morin, Amenos, Alsina and Gorina, 1982). Captopril has an antihypertensive effect in anephric patients (Vaughan, Carey, Ayers and Peach, 1979). This shows that captopril can reduce blood pressure in man, independantly of circulating renin and AII concentrations. In rats, after nephrectomy, despite the absence of renin in the peripheral circulation, captopril still produces a fall in blood pressure. Captopril also produces a substantial fall in blood pressure in rats with late Goldblatt, 2-kidney, 1-clip hypertension and in rats made hypertensive by the chronic administration of deoxycorticosterone and a high salt diet, (Malik and Nasjletti, 1976). Both these models of hypertension are believed to be renin independant. In rats in which the R-AII system has been blocked by saralasin, captopril also produces a further reduction in blood pressure (Thurston and Swales, 1978). So it would appear that the effectiveness of captopril is not solely dependant on its ability to to reduce elevated circulating levels of AII as originally assumed.

There is evidence that AII regulates CA release from the adrenal medulla, and removal of this regulation by captopril could be important. Insulin hypoglycaemia, hypoxia, hypercapnia, haemorrhage, cold shock and muscular exercise all stimulate CA release from the adrenal medulla (see Callingham, 1975 for review). This compensatory release of CAs is important in restoring homeostasis after such shocks to the cardiovascular system, and it follows that any drug interfering with such a release of CAs from the adrenal medulla could greatly impair this necessary compensatory mechanism. The importance of CA

secretion from the adrenal medulla in response to hypotensive activation of the carotid sinus reflex was first demonstrated by Bedford and Jackson in 1916 and Heymans in 1929. Both chemoreceptor stimulation (Petropaulovskaya, 1953) and baroreceptor stimulation (Anichkov, Malyghina, Poskalenko and Ryzhenkov, 1960) were subsequently discovered to induce release of CAs from the adrenal medulla. Many studies have since proved that there is an adrenomedullary secretion of CAs following haemorrhage (Millar and Benfey, 1958; Greever and Watts, 1959; Chiens, 1967; Feuerstein and Gutman, 1971; Adamicza, Tarnoky and Nagy, 1980; England, Dempsher, Byrnes, Presnell and Gann, 1981), carotid occlusion (Guazzi, Libretti and Zanchetti, 1962; Oberg and White, 1970) and a specific lowering of carotid perfusion pressure (Critchley etal, 1980).

Tobian (1965) first suggested there was an activation of the R-AII system in hypovolemic and hypotensive states. In 1971, Peach demonstrated that AII stimulates the release of CA from isolated adrenal glands, and there is much evidence to suggest that AII stimulates sympathetic ganglion cells and the adrenal medullary secretion of CAs in vivo (see Starke, 1972; Haefely, 1972; Reit, 1972 for reviews). AII has also been shown to depolarise chromaffin cells and stimulate CA release from the chromaffin cells in a calcium-dependant manner (Poisner and Douglas, 1966).

Circulating AII levels increase after haemorrhage in cats (Feuerstein etal, 1977) and dogs (Harrison, Birbari and Seaton, 1973) and blockade of the R-AII system inhibits the adrenomedullary response to haemorrhage in cats and dogs (Harrison etal, 1973). Feuerstein etal

(1977) showed that in the cat, blockade of the R-AII system by bilateral nephrectomy or saralasin, and denervation of the adrenal gland inhibits the release of CAs from the adrenal medulla in response to haemorrhage. They concluded that this was due to a central activation of sympathetic drive by AII (see later).

There is also much evidence to suggest that AII may facilitate the activity of the splanchnic nerve and its subsequent stimulation of adrenal medullary CA release. This is suggested by studies showing that AII potentiates the stimulated release of NA by peripheral sympathetic nerves and sympathetic ganglion cells. (The evidence for this will be reviewed in detail in Part III of this thesis). It is also evident that AII potentiates vasoconstriction induced by sympathetic nerve activity. AII potentiates the vasoconstriction induced by sympathetic nerve stimulation in the rat mesenteric vascular bed at doses which do not have a direct vasoconstrictor effect (Clough, Collis, Conway, Hatton and Keddie, 1982). Captopril inhibits the vascular response to NA in this preparation (Saruta, Suzuki, Okimo and Kondo, 1982). Saralasin and captopril both reduce the pressor responses to NA in the pithed rat and this effect is abolished by nephrectomy (Clough etal, 1982) and captopril inhibits pressor responses to peripheral sympathetic nerve activity in cats (Boura, Hui, Rechtman and Walters, 1982). AII not only enhances cardiovascular responses to sympathetic nerve activity (Page, Kaneko and McCubbin, 1966) but also those which follow excitation of sympathetic ganglion stimulants (McCubbin and Page, 1963; Day and Owen, 1970; Boura etal, 1982). The evidence indicates that inhibitors of the R-AII system may impair vasoconstriction by interfering with

both pre-and post-junctional action of AII, and this demonstrates a functional interaction between the R-AII system and sympathetic nervous control of vasomotor tone which exists even when AII levels are not elevated. Various "non-AII" actions of captopril have also been suggested, involving interactions with prostaglandins, bradykinin, opioid peptides and adrenocorticosteroids. These interactions were investigated in this project and will be discussed in detail in the relevant Parts (II-V) of this thesis.

It is apparent from the evidence discussed that the hypotensive action of captopril does not depend only on its ability to reduce elevated circulating AII and the resultant central stimulation of sympathetic drive by peripheral and/or central AII.

This thesis is concerned primarily with the possible interactions of captopril and AII, on the reflex release of CAs from the adrenal medulla following a lowering of carotid perfusion pressure (subsequently refered to as "baroreceptor stimulation").

In summary AII appears to facilitate the release of CAs from the adrenal medulla either by a direct action on adronomedullary cells, or indirectly via interactions with prostaglandins, opioid peptides, bradykinin , adrenocorticosteroid secretion and the central nervous system. AII may also facilitate release of CA from the adrenal medulla via an action on splanchnic nerve activity, and it has been shown to facilitate other cardiovascular effects of the sympathetic nervous system. Captopril, by reducing circulating AII levels, could reduce CA output from the adrenal medulla and sympathetic drive by a combination

of mechanisms.

It is the aim of this project to investigate the possible interactions of the R-AII system on both the basal output (subsequently refered to as the resting release or output), and the reflex release induced by baroreceptor stimulation, of CAs from the adrenal medulla.

It is important to understand the extent to which captopril and other drugs which interfere with the R-AII system impair the physiological compensatory mechanisms which protect the body from the types of cardiovascular stresses previously discussed. An understanding of this would assist in the clinical prediction and management of patients receiving drugs which interfere with the R-AII system.

As has been mentioned, Feuerstein etal (1977) demonstrated that, in the anaesthetised cat, haemorrhage induced release of CAs from the adrenal medulla and this release was accompanied by an increase in PRA. The CA release was inhibited by both nephrectomy and saralasin administration. They concluded that the release of CAs was due to activation of the renin-AII system, and AII was acting centrally to increase sympathetic drive, and hence adrenal CA release. In the light of evidence that AII has a direct facilitatory effect on adrenal CA release, it seemed possible that the effect of AII, following baroreceptor stimulation could be at the level of the gland itself, rather than at the level of the central nervous system. In their experiments, Feuerstein etal (1977) began analysing CA release 10

minutes after the onset of haemorrhage. it was unclear from their data whether there was a significant increase in PRA up to 10 minutes post haemorrhage. It was therefore of interest to investigate whether or not there was an immediate release of catecholamines 1-10 minutes after the onset of baroreceptor stimulation, and if so, if this release was related to an increase in PRA.

The aims of the experiments in Part I of this thesis were primarily to answer the following questions-:

1. Does captopril reduce the resting output of CAs from the adrenal medulla, and/or the reflex release of CAs during a 10 minute baroreceptor stimulation period ?

2. If so, is this inhibitory effect of captopril related to any increase in plasma renin activity and can the inhibition be reversed by exogenously administered AII ?

Introduction to results

In all tables and figures, "n" refers to the total number of baroreceptor tests performed and "total catecholamine release" refers to the release of adrenaline and noradrenaline. Where "n" values are omitted from figures, they can be found in the tables from which they were compiled. Where statistical significance values are omitted from figures, these can also be found in the relevant tables, and vice versa. Statistical significance of results was assessed by the paired t-test when $n > 5$. If no statistics are shown and $n > 5$, then statistical analysis showed the results to be not significant ($p > 0.05$). Linear regression lines with 95% confidence limits were constructed using a computer program.

In the tables where two values are given, separated by a comma (i.e. see table 1.1), this refers to the two values obtained in separate baroreceptor tests or splanchnic nerve stimulations. "Resting release" or "resting output" of catecholamines refers to the release prior to either baroreceptor or splanchnic nerve stimulation. "Change in total catecholamine release" refers to the release of catecholamines during baroreceptor or splanchnic nerve stimulation minus the resting release of catecholamines, i.e. the reflex or stimulated release. Systemic blood pressure is always expressed in mmHg.

In all experiments, I refer to drugs being administered "after" or "following" captopril. It should be noted that these drugs were effectively administered with captopril, as the effects of captopril

outlast the duration of any experiment.

Throughout the results sections I shall include "comments" on the results given. Additional discussion shall be given in the "discussion" section which follows each "results" section.

Part 1 - Results

1.A. The effect of captopril on release of catecholamines before and after baroreceptor stimulation from the left adrenal gland of the anaesthetised dog

In dogs 1-9, ten-minute baroreceptor tests were performed before and after captopril administration. The effect of captopril on total catecholamine release, before and after baroreceptor stimulation was analysed. The results are shown in table 1.1, and illustrated in figure 1.1.

The effect of captopril on the reflex increase in total catecholamine release following baroreceptor stimulation was also analysed and the results are shown in table 1.2 and figure 1.2.

The results show that there was a significant increase in total catecholamine release 1-2, 5-6 and 9-10 minutes after the onset of, and during, baroreceptor stimulation. Following captopril administration, both the resting release of catecholamines and the reflex release induced by baroreceptor stimulation were significantly inhibited.

1.B. The effect of captopril on release of catecholamines before and after baroreceptor stimulation from the left adrenal gland of the anaesthetised cat

In three cats, ten minute baroreceptor tests were performed before

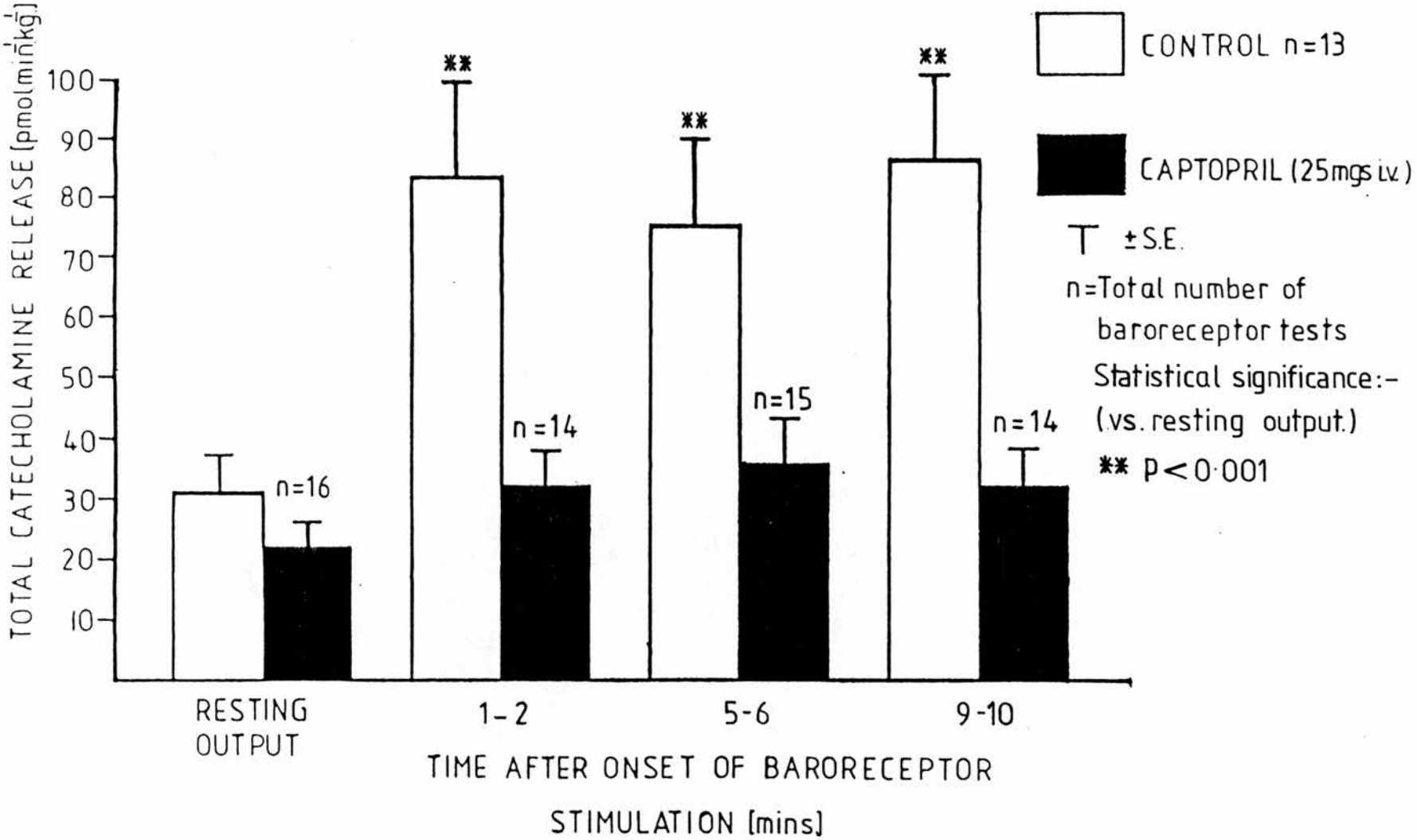
Table 1.1

The effect of captopril on total catecholamine release in dogs 1-9 before and after baroreceptor stimulation (BRS).

Drug Treatment	Time after onset of BRS (mins)	Total catecholamine release ($pmolmin^{-1}kg^{-1}$) D1	D2	D3	D4	D5	D6	D7	D8	D9	Mean ±SE
Control	0	20,28	16,22	30,53	8	35	26	15,14	78	61	31.2 ± 5.7 (n=13)
	1-2	44,38	84,100	99,147	16	66	54	35,24	217	153	82.8 ± 16.5 (n=13)
	5-6	52,42	54,72	103,114	24	65	66	28,13	203	137	74.8 ± 14.6 (n=13)
	9-10	62,52	70,130	147,155	24	78	92	19,29	169	89	85.8 ± 14.1 (n=13)
Captopril (25mg)	0	14,0	10,10	18,12	12	34	32,24	10,14	67,48	24,21	21.9 ± 4.2 (n=16)*
	1-2	16,0	24,24	77,52	8	38	38,38	19,14	58	40	31.9 ± 5.6 (n=14)*
	5-6	18,0	38,8	58,73	8	58	24,36	15,14	98	28,55	35.4 ± 7.2 (n=15)**
	9-10	22,0	30,16	65	10	58	26,40	13,16	63	29,57	31.8 ± 5.7 (n=14)**

Statistical significance (captopril data compared with control data)-:
* = $p < 0.01$
** = $p < 0.001$

Figure 1·1



The effect of captopril on total catecholamine release, before and after baroreceptor stimulation, in dogs 1-9.

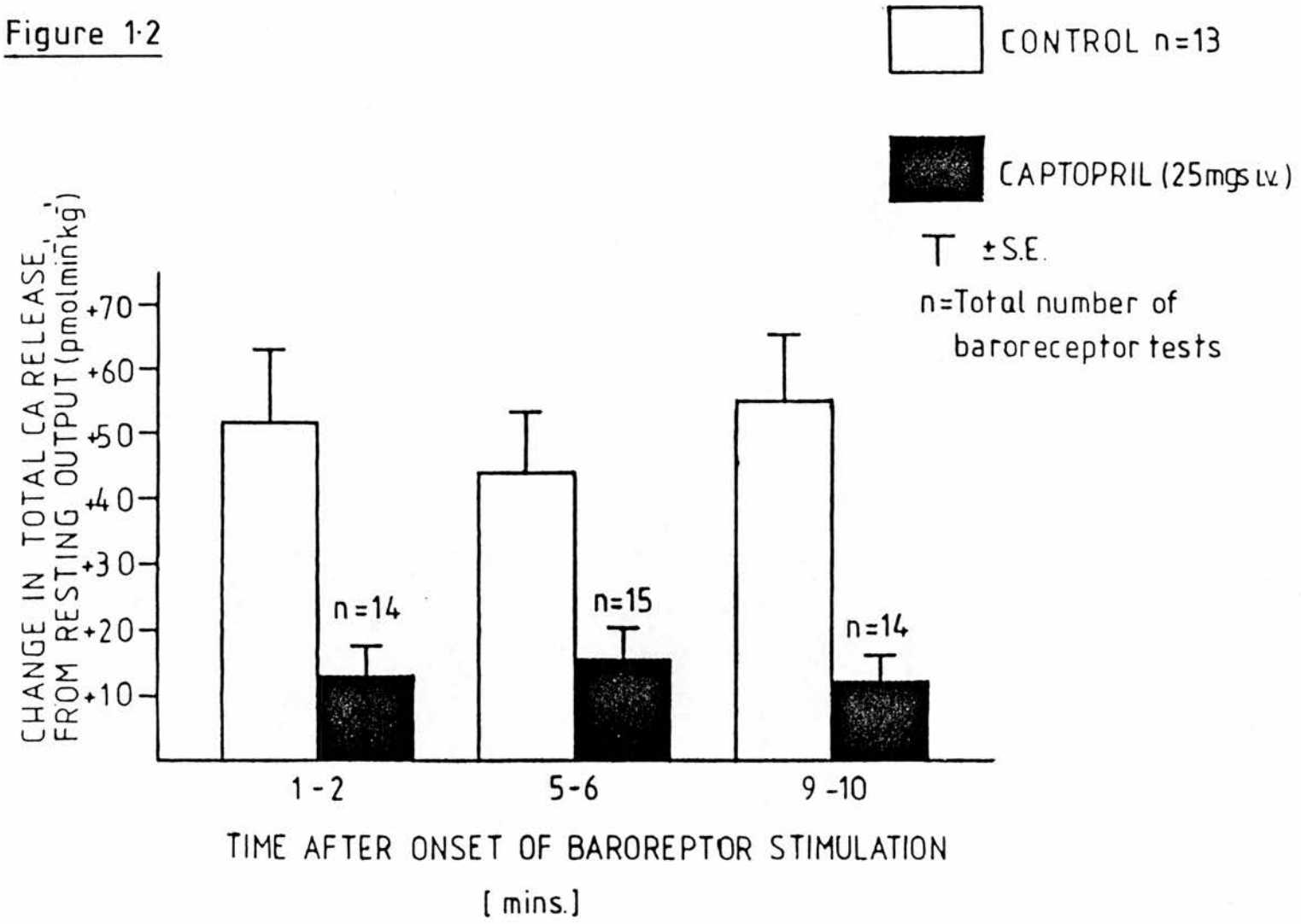
Table 1.2

The effect of captopril on the change in catecholamine release induced by baroreceptor stimulation (BRS) in dogs 1-9 (D1-9).

Drug Treatment	Time after onset of BRS (mins)	Change in catecholamine release ($pmolmin^{-1}kg$) D1	D2	D3	D4	D5	D6	D7	D8	D9	Mean ±SE
Control	0										
	1-2	24,10	68,78	69,94	8	31	28	20,10	139	92	51.6 ± 11.4 (n=13)
	5-6	32,14	38,50	73,61	16	30	40	13.-1	125	76	43.6 ± 9.4 (n=13)
	9-10	42,24	54,108	117,102	16	43	66	4,15	9	28	54.6 ± 10.7 (n=13)
Captopril (25mg)	0										
	1-2	2,0	14,14	59,40	-4	4	6,14	9,0	10	16	13.1 ± 4.5 (n=14)*
	5-6	-14,0	28,-2	40,61	-4	24	-8,12	5,0	31	4,34	15.3 ± 5.2 (n=15)**
	9-10	8,0	20,6	47	-2	24	-6,16	3,2	15	5,36	12.4 ± 4.0 (n=14)**

Statistical significance (captopril data compared with control data)-:
* = $p < 0.01$
** = $p < 0.001$

Figure 1·2



The effect of captopril on the change in total catecholamine release, following baroreceptor stimulation, in dogs 1-9.

and after captopril administration. The effect of captopril on total catecholamine release, before and after baroreceptor stimulation was analysed. The results are shown in table 1.3.

The effect of captopril on the reflex increase in catecholamines was also analysed. The results are shown in table 1.4.

The results show that there is an increase in total catecholamine release 1-2, 5-6 and 9-10 minutes after the onset of, and during, baroreceptor stimulation. Following captopril administration, the reflex release of catecholamines was inhibited.

2.A. Plasma renin activity before and after baroreceptor stimulation in the anaesthetised dog

In dogs 1-7, arterial plasma renin activity (PRA) was measured prior to baroreceptor stimulation, and 1-2, 5-6 and 9-10 minutes after the onset of, and during, baroreceptor stimulation, before and after captopril administration. The results are shown in table 1.5 and illustrated in figure 1.3.

The results show that during baroreceptor stimulation there was no significant increase in PRA. Figure 5 shows that captopril significantly increased PRA prior to baroreceptor stimulation, and levels remained elevated throughout the baroreceptor test. This is probably due to captopril abolishing the negative feedback system that normally exists, angiotensin II inhibiting further renin release (see discussion). After captopril administration there was no increase in

Table 1.3

The effect of captopril on total catecholamine release in cats 1-3 before and after baroreceptor stimulation (BRS).

Drug Treatment	Time after onset of BRS (mins)	Cat1	Cat2	Cat3	Mean ± SE
Control	0	32,78	40,98	44	58.4 ± 12.6
	1-2	82,120	136,200	90	125.6 ± 21 **
	5-6	58,134	84	184	115.0 ± 27.8*
	9-10	72,112	92	116	98.0 ± 10.2**
Captopril (5mg)	0	15,75	56,96	46,57	57.5 ± 11.2
	1-2	91	69,113	59,93	85.0 ± 9.5 *
	5-6	35	70,125	59,71	72.0 ± 14.8
	9-10	41	61,144	53,44	69.6 ± 19.2

Statistical significance (Control release during BRS compared with control release before BRS and captopril data compared with control data)-:

* = $p < 0.05$

** = $p < 0.01$

Table 1.4

The effect of captopril on the change in catecholamine release induced by baroreceptor stimulation (BRS), in cats 1-3.

Drug Treatment	Time after onset of BRS (mins)	Change in catecholamine release ($pmolmin^{-1}kg^{-1}$)			Mean ± SE
		Cat1	Cat2	Cat3	
Control	0				
	1-2	50,42	92,102	46	67.2 ± 13
	5-6	26,56	44	140	66.6 ± 25.2
	9-10	40,36	52	72	50 ± 8.0
Captopril (5mg)	0				
	1-2	16	13,17	13,36	19 ± 4.3 *
	5-6	20	14,29	13,14	18 ± 3.0
	9-10	26	5,48	7,-13	14.6 ± 10.4**

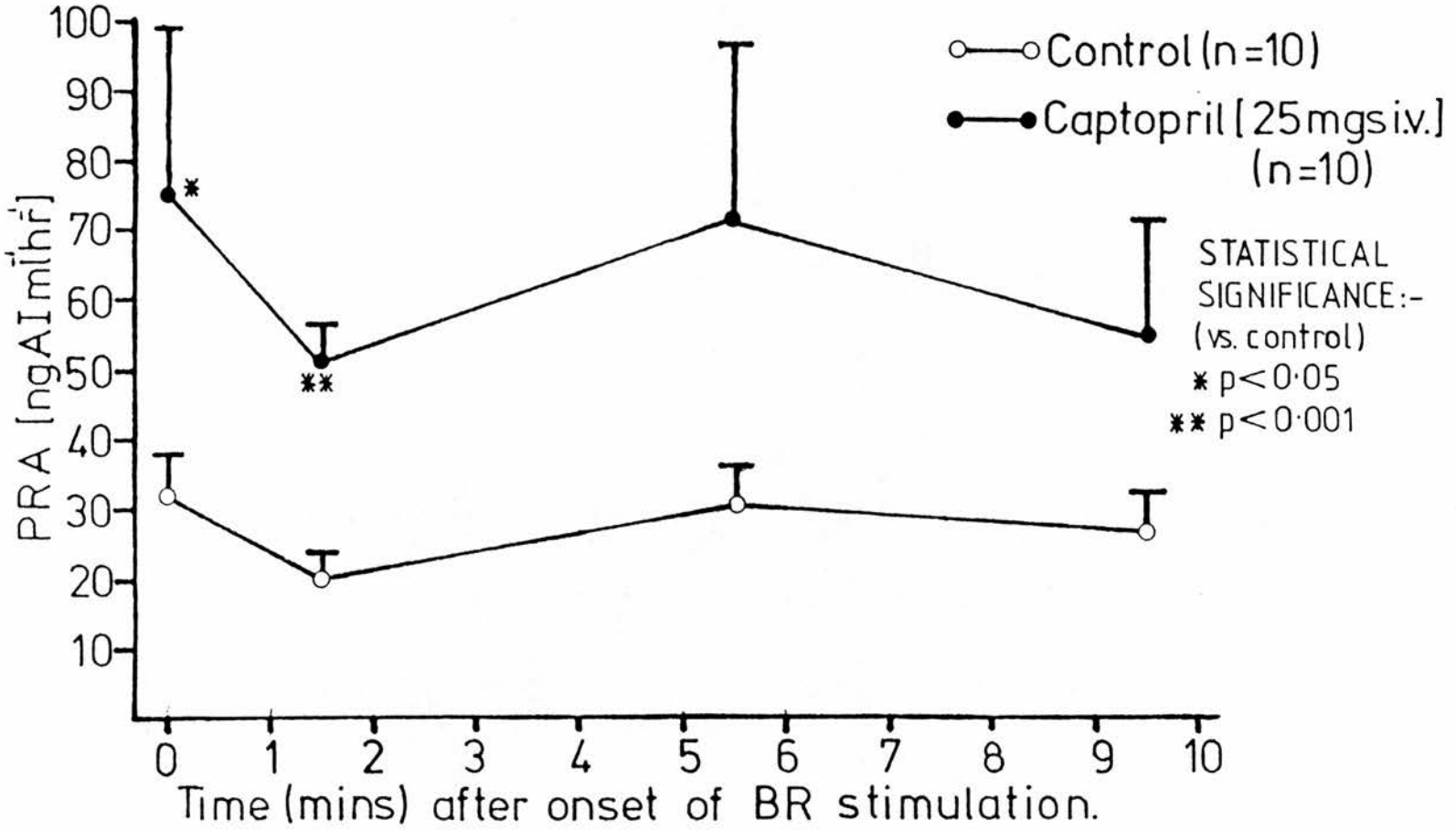
Statistical significance (captopril data compared with control data)-:
* = $p < 0.05$
** = $p < 0.02$

Table 1.5

Plasma renin activity in dogs 1-7 (D1-7) before and after baroreceptor stimulation (BRS).

Drug Treatment	Time after onset of BRS (mins)	Plasma renin activity ($ngAIml^{-1}hr^{-1}$)							Mean ± SE (n=10)
		D1	D2	D3	D4	D5	D6	D7	
Control	0	31	48,54	56	13	33,39	37	4,6	32.1±6.0
	1-2	18	35,16	32	34	12,29	11	4,14	20.5±3.5
	5-6	30	58,54	58	33	13,31	10	4,13	30.4±6.5
	9-10	34	54,38	58	17	17,17	17	6,9	26.7±5.8
Captopril (25mg)	0	64,30	144	272	44,34	38	50,43	33	75.2±24.3
	1-2	76,34	51	82	43,35	45	56,58	32	51.2±5.4
	5-6	74,37	42	288	63,26	41	69,29	42	71.0±24.7
	9-10	86,30	16	192	43,30	29	52,32	34	54.4±16.4

Figure 1·3



Plasma renin activity [PRA] in dogs 1-7 before and after baroreceptor stimulation

PRA during baroreceptor tests, in fact there was a significant reduction in PRA 1-2 minutes after the onset of baroreceptor tests.

2.B. PRA before and after baroreceptor stimulation in the anaesthetised cat

In cats 1-3, arterial PRA was measured prior to baroreceptor stimulation, and 1-2, 5-6 and 9-10 minutes after the onset of, and during, baroreceptor stimulation. The results are shown in table 1.6.

The results show that there was no increase in PRA during baroreceptor stimulation. Unlike the dog there was no increase in PRA following captopril administration. This may not have come to light due to the small number of samples analysed. I was informed that PRA in cat 3 was very difficult to measure as it was at the minimum level of the radioimmunoassays sensitivity. If more experiments had been possible, perhaps an increase in PRA following captopril administration would have been uncovered.

Comments

25mg (dogs) and 5mg (cats) of captopril was the dose of choice as it has been shown to completely inhibit PRA and in these experimental animals it completely inhibited the pressor response to doses of angiotensin I.

The results shown in sections 1 and 2 of part 1 show that there was a significant increase in catecholamine release 1-2, 5-6 and 9-10 minutes after onset of baroreceptor stimulation. This increase was not

Table 1.6

Plasma renin activity in cats 1-3 before and after baroreceptor stimulation (BRS).

Drug Treatment	Time after onset of BRS (mins)	Plasma renin activity (ng AIml^{-1}hr^{-1})			
		Cat1	Cat2	Cat3	Mean ± SE
Control	0	56,40	17,24	4,8	23.8 ± 8.2
	1-2	61	19,12	8	25.0 ± 12.2
	5-6	16,32	18,26	24	23.2 ± 2.9
	9-10	74	19	16,20	32.3 ± 13.9
Captopril (5mg)	0	24,32	48,12	3	23.8 ± 7.8
	1-2	24	16,21	-	20.3 ± 2.3
	5-6	40,40	18,8	5	22.2 ± 7.6
	9-10	16,32	12,12	8	16.0 ± 4.2

accompanied by an increase in PRA. This makes it unlikely that it is an increase in circulating angiotensin II which is responsible for the reflex release of catecholamines, as was suggested by Feuerstein etal (1977).

The results also show that there was a significant increase in catecholamine release 1-2 minutes after baroreceptor stimulation. This immediate response makes it unlikely that de novo synthesised angiotensin II was acting centrally to increase sympathetic drive and thereby stimulating the reflex release of catecholamines from the adrenal medulla, as suggested by Feuerstein etal (1977). We suggest that if such a mechanism did exist there would be evidence of an increase in PRA and reflex catecholamine release would be delayed.

We took as a working hypothesis, at this stage of the research, that it was a minimum circulating level of angiotensin II which was the essential criterion for the adrenal gland to respond to the reflex stimuli. Removal of this minimum level of angiotensin II by captopril rendered the gland unable to respond fully to the reflex stimuli. We hypothesised that angiotensin II was exerting a permissive effect on adrenal catecholamine release at the level of the gland itself and not centrally.

3.A. The effect of a continuous i.v. infusion of angiotensin II on adrenal catecholamine release before and after baroreceptor stimulation, following captopril administration, in the anaesthetised dog

We wished to investigate if restoring a very low, non pressor

level of angiotensin II (AII) could restore the release of catecholamines which captopril had inhibited.

In dogs 1-5, following captopril administration, a continuous infusion of 1-5ngmin^{-1} AII was administered and the baroreceptor tests repeated.

The effect of this infusion of AII on total catecholamine release was analysed and the results are shown in table 1.7 and illustrated in figure 1.4. Figure 1.4 was compiled from the data contained in tables 1.1 and 1.7.

The results show that the AII partially restored both the resting release of catecholamines and also the reflex release. When comparing the release of catecholamines after AII infusion with the releases after captopril administration, AII can be seen to reverse the inhibition by captopril, although not completely.

The effect of AII on the changes in total catecholamine release following baroreceptor stimulation was also analysed. The results are shown in table 1.8 and illustrated in figure 1.5.

The results show that AII partially reversed the inhibition, by captopril, of the reflex induced catecholamine release.

Table 1.7

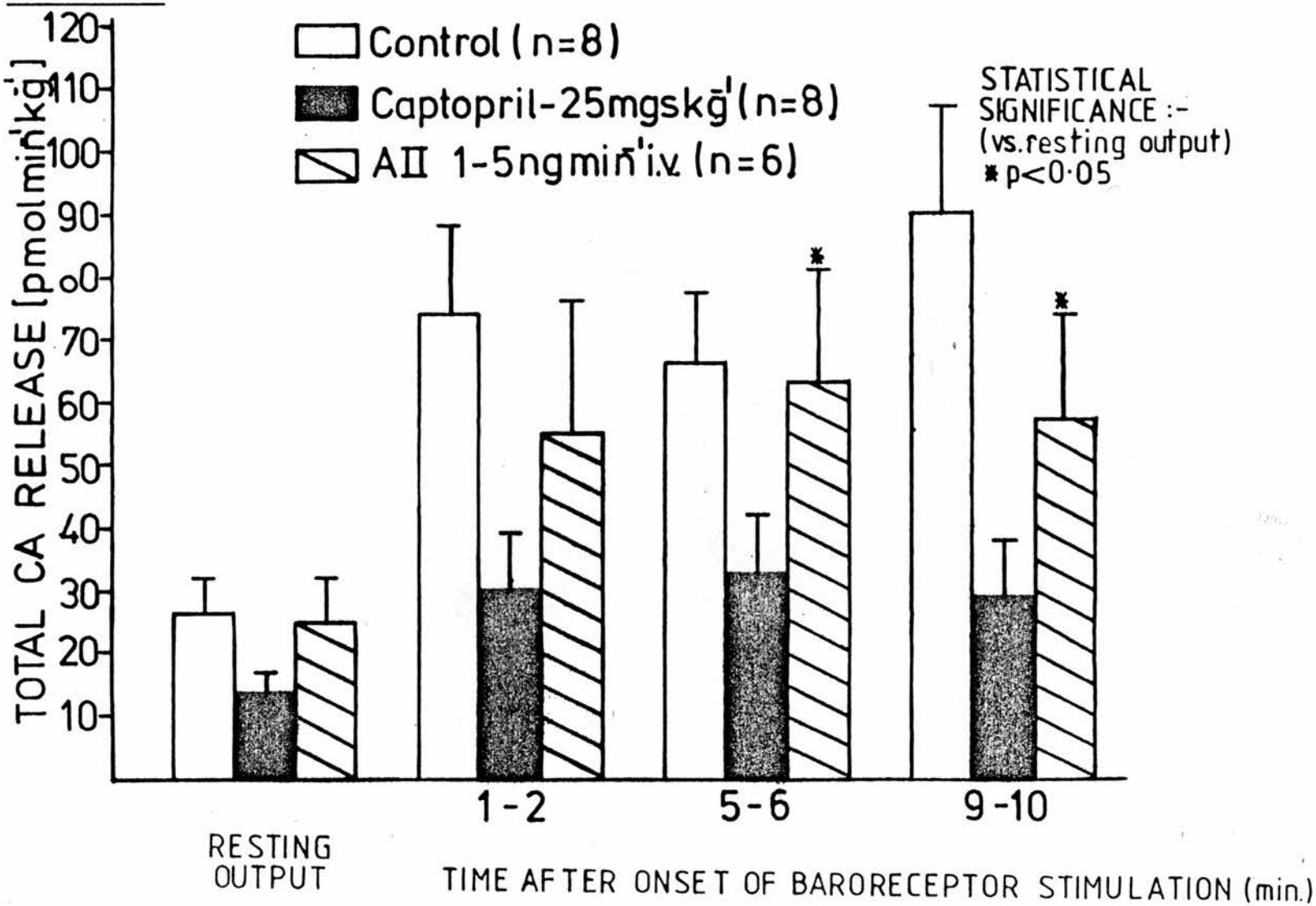
The effect of a continuous infusion of 5ngmin^{-1} angiotensin (AII), following captopril administration, on adrenal catecholamine release in dogs 1-5

Drug Treatment	Time after onset of BRS (mins)	Total catecholamine release (pmolmin^{-1}kg^{-1})					
		Dog1	Dog2	Dog3	Dog4	Dog5	Mean (n=6)
AII + Captopril	0	14	18	15,58	12	34	25.2 ± 7.3
	1-2	26	40	53,160	14	35	54.7 ± 21.7
	5-6	20	36	96,120	18	90	*63.3 ± 17.9
	9-10	28	40	36,120	22	97	57.2 ± 16.7
							(n=8)
Captopril	0	(see table 1 for values)					13.8 ± 3.4
	1-2						29.9 ± 9.9
	5-6						32.6 ± 9.9
	9-10						28.7 ± 9.2

Statistical significance (AII data compared with captopril data)-:
* = $p < 0.01$

The captopril "mean" data given here was calculated from the values contained in table one, for dogs 1-5. It has been included in order to compare the effect of administration of AII, following captopril administration, with that of captopril alone.

Figure 1·4



The effect of a continuous infusion of angiotensin II (AII), after captopril administration, on total catecholamine (CA) release, before and after baroreceptor stimulation.

Table 1.8

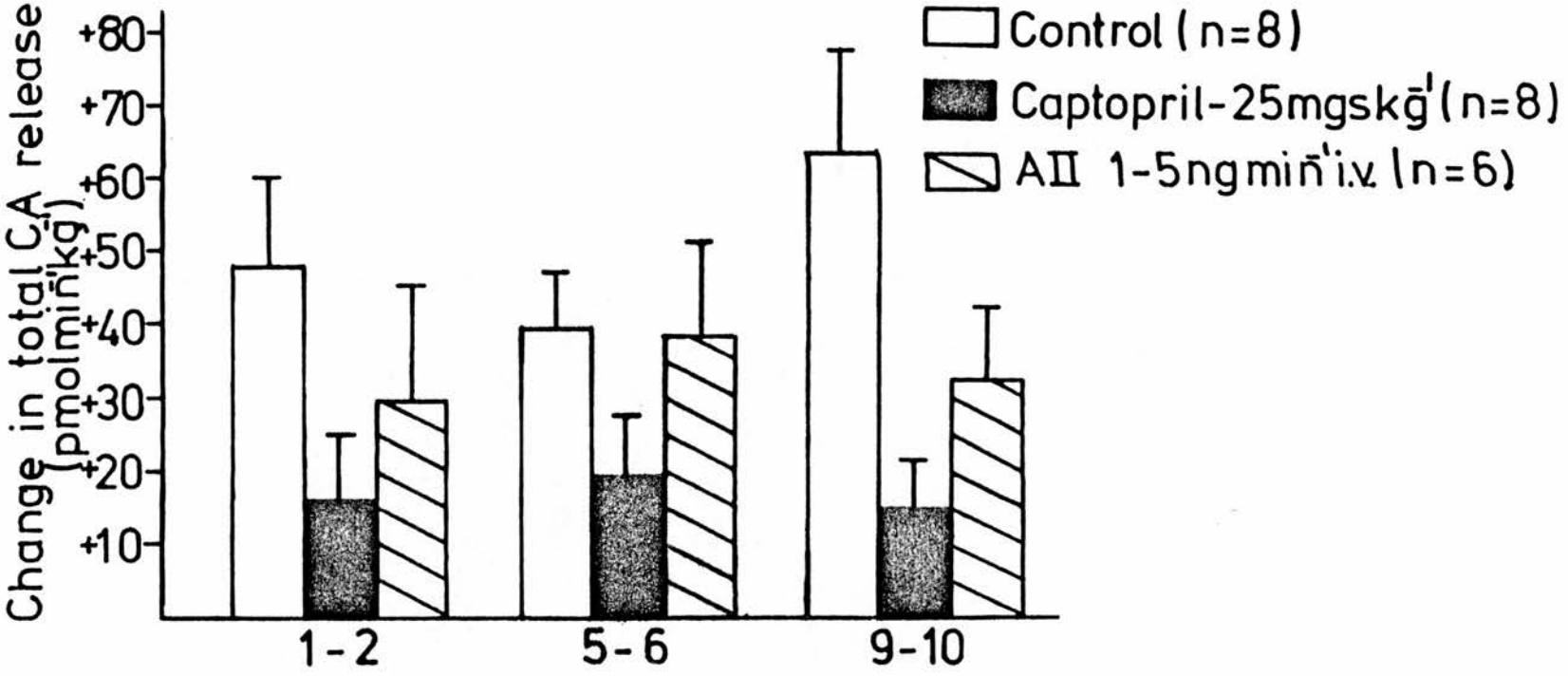
The effect of a continuous infusion of 5ngmin^{-1} angiotensin (AII), following captopril administration, on the change in adrenal catecholamine release, induced by baroreceptor stimulation (BRS) in dogs 1-5 (D1-5).

Drug Treatment	Time after onset of BRS (mins)	Change in catecholamine release (pmolmin^{-1}kg^{-1})					
		Dog1	Dog2	Dog3	Dog4	Dog5	Mean (n=6)
	0						
AII	1-2	12	22	38,102	2	1	29.5 ± 15.5
+	5-6	6	18	81,62	6	56	38.2 ± 13.2*
Captopril	9-10	14	22	21,62	10	63	32.0 ± 9.8
							(n=8)
	0						
Captopril	1-2	(see table 2 for values)					16.1 ± 7.8
	5-6						16.6 ± 9.1
	9-10						14.7 ± 6.5

Statistical significance (AII data compared with captopril data)-:
* = $p < 0.02$

The captopril "mean" data given here was calculated from the values contained in table two, for dogs 1-5. It has been included in order to compare the effect of administration of AII, following captopril administration, with that of captopril alone.

Figure 1·5



The effect of a continuous infusion of angiotensin II (AII), after captopril administration, on the change in total catecholamine (CA) release, before and after baroreceptor stimulation.

3.B. The effect of a continuous infusion of AII on adrenal catecholamine release before and after baroreceptor stimulation, following captopril administration, in the anaesthetised cat

In cats 1-3, following captopril administration, a continuous infusion of 1-5ngmin^{-1} AII was administered and the baroreceptor tests repeated. The effect of AII on total catecholamine release and the reflex changes in catecholamine release were analysed. The results are shown in tables 1.9a and 1.9b and illustrated in figure 1.6. The results show that AII reversed the inhibition by captopril of both the resting and reflex releases of catecholamines. In figure 1.6 and subsequent figures the "increment" refers to total catecholamine release during baroreceptor stimulation minus resting release.

Comments

It will have been noticed that there is a great variation between animals in the values of total catecholamine release. This has resulted in very large standard errors in most of the data I analysed. Many studies have quoted a 10-30 fold difference in baseline adrenal catecholamine release between different animals (see Heesch and Bishop, 1982 for references). The differences probably arise from differences in condition, anaesthesia and surgical preparations etc. which occur between whole animal preparations. This was a problem that could not be avoided.

The results described in section 3 support our initial hypothesis that a minimum level of circulating AII is required for the adrenal gland to respond to the reflex stimuli. Removal of this AII inhibits,

Table 1.9a

The effect of a continuous infusion of 5ngmin^{-1} angiotensin (AII), following captopril administration, on adrenal catecholamine release in cats 1-3.

Drug Treatment	Time after onset of BRS (mins)	Total catecholamine release (pmolmin^{-1}kg^{-1})			
		Cat1	Cat2	Cat3	Mean ± SE
AII + Captopril	0	444,284	100,209	80,90	201.2 ± 58.7
	1-2	464	166,258	123,123	226.8 64.2
	5-6	511,594	186,264	110,122	298.0 84.3
	9-10	498	206,309	136,60	241.0 76.0
Captopril	0	(See table 3 for values)			58.4 ± 12.6
	1-2				125.6 ± 12.6
	5-6				115.0 ± 27.8
	9-10				98.0 ± 10.2

The captopril "mean" data given here was calculated from the values contained in table 1.3 , for cats 1-3. It has been included in order to compare the effect of administration of AII, following captopril administration, with that of captopril alone.

Table 1.9b

The effect of a continuous infusion of 5ngmin^{-1} angiotensin (AII), following captopril administration, on the change in total catecholamine release in cats 1-3.

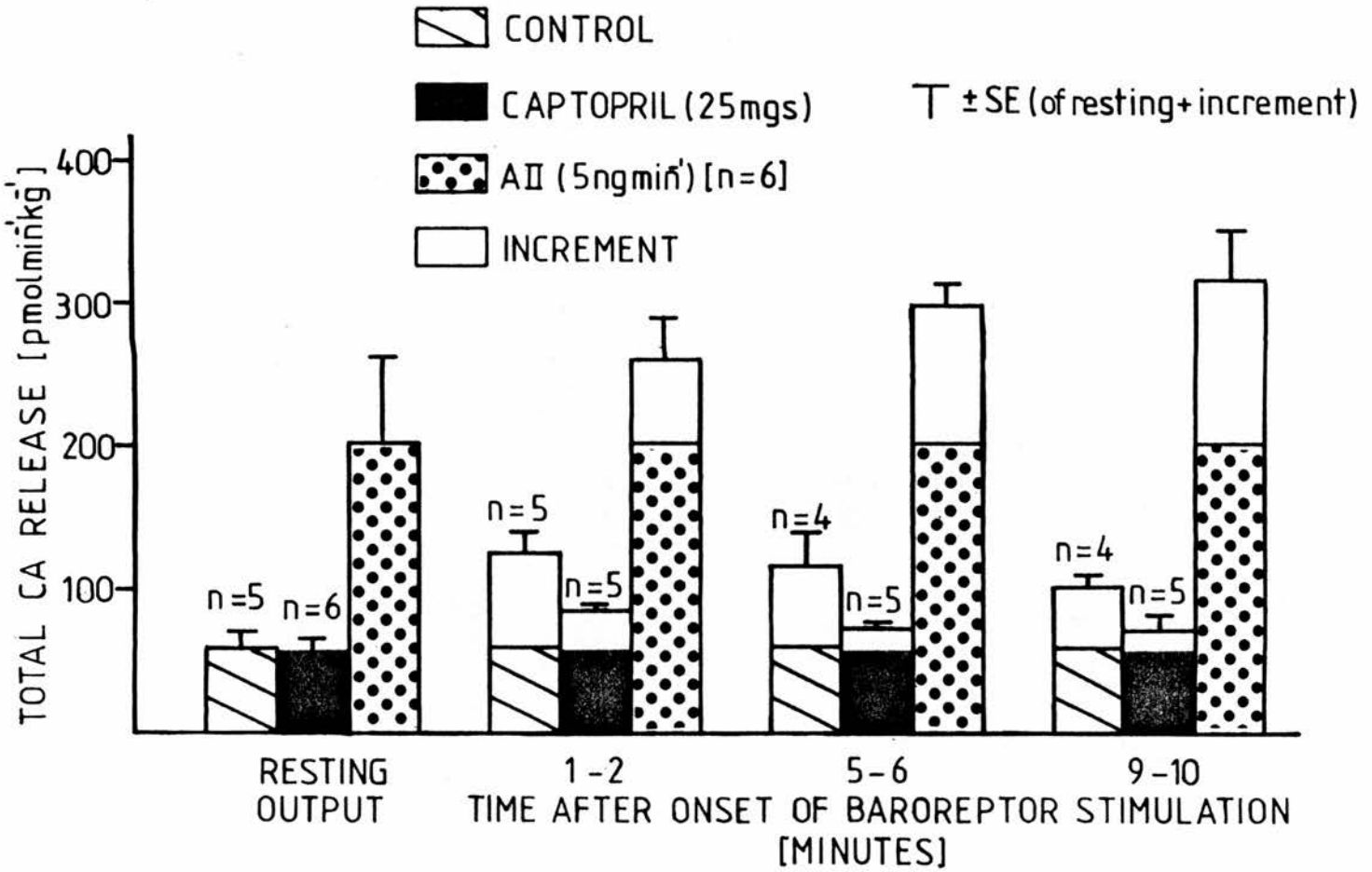
Drug Treatment	Time after onset of BRS (mins)	Change in total catecholamine release (pmolmin^{-1}kg^{-1})			
		Cat1	Cat2	Cat3	Mean ± SE
AII + Captopril	0				
	1-2	180	66,49	43,33	74.2 ± 27.0
	5-6	67,310	86,55	30,32	96.7 ± 43.5*
	9-10	54	106,200	56	104.0 ± 34.2*
Captopril	0				
	1-2	(See table 4 for values)			67.2 ± 13.0
	5-6				66.6 ± 23.2
	9-10				50.0 ± 8.0

Statistical significance (AII data compared with captopril data)-:
* = $p < 0.05$

The captopril "mean" data given here was calculated from the values contained in table 1.4 , for cats 1-3. It has been included in order to compare the effect of administration of AII, following captopril administration, with that of captopril alone.

Figure 1·6

The effect of captopril(i.v.) followed by a continuous i.v. infusion of angiotensin II (AII) on total catecholamine [CA] release, before and after baroreceptor stimulation in cats 1-3.



and subsequent replacement restores, catecholamine release.

The concentration of AII used in these experiments was very low and had no effect on systemic blood pressure (see following results section).

4.A. The effect of captopril and subsequent AII administration on systemic blood pressure, before and after baroreceptor stimulation in the anaesthetised dog

The effect of captopril on resting systemic blood pressure (SBP), and the reflex increase in SBP induced by baroreceptor stimulation, was analysed in dogs 1-9. The results are shown in table 1.10 and illustrated in figures 1.7a and 1.7b. In dogs 1-5 the effect of the AII infusion on SBP was also analysed. The results are included in table 1.10.

The results show that captopril significantly decreased resting SBP and the reflex pressor response to baroreceptor stimulation.

The results show that the concentration of AII infused did not increase either resting SBP or the reflex pressor response. From table 1.10 it would appear that AII further decreased resting SBP, when compared with the resting SBP immediately preceeding (after captopril) and the reflex pressor response. This was probably not due to an effect of AII but to the fact that AII was administered some six hours after anaesthesia was first induced. At this stage of most experiments, SBP was beginning to fall anyway, due to the length of

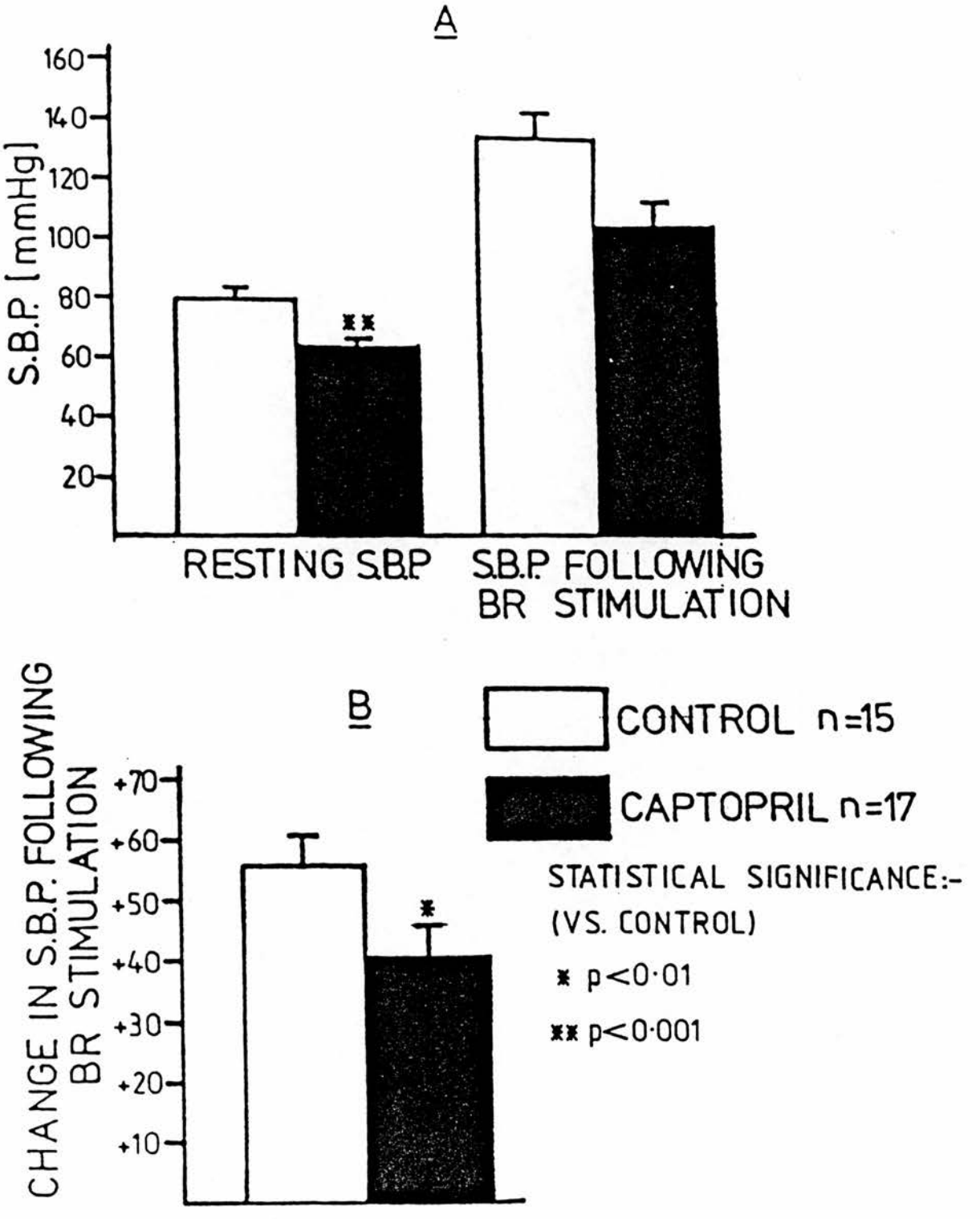
Table 1.10

The effect of captopril on resting systemic blood pressure (SBP) and the reflex increase in SBP following baroreceptor stimulation (BRS), in dogs 1-9.

Dog number	Resting SBP			SBP following BRS			Increment		
	Control	Captopril	AII	Control	Captopril	AII	Control	Captopril	AII
1	85,85	55,65	65,45	115,105	65,80	80,75	30,20	10,15	15,30
2	60,50	50,50	45,40	120,80	80,80	70,90	60,35	30,30	25,50
3	85,70	60,50	60,60	130,110	75,70	90,85	45,40	15,15	30,25
4	70	60,65	70,55	130	90,110	120,110	60	30,45	50,55
5	85,70	40,55	45,35	170,110	65,120	60,70	85,40	25,65	15,35
6	60	70		140	145		80	75	
7	100,110	90,90		195,200	170,170		95,90	80,80	
8	90,80	75,70		150,120	125,120		60,40	50,50	
9	90	70,75		140	100,120		50	30,45	
Mean ± SE	79.5 ± 4.1 (n=15)	**64.4 ± 3.3 (n=17)	52.0 ± 3.7 (n=10)	134.7 ± 8.4 (n=15)	105.0 ± 8.3 (n=17)	85.0 ± 5.9 (n=10)	55.3 ± 6.0 (n=15)	*40.6 ± 5.7 (n=17)	33.0 ± 4.5 (n=10)

Statistical significance (captopril data compared with control data)-:
* = $p > 0.01$
** = $p > 0.001$

Figure 1·7



The effect of captopril on systemic blood pressure (S.B.P.) before and after baroreceptor (BR) stimulation (A) and on the pressor response to BR stimulation (B).

time the preparation had been in use.

4.B. The effect of captopril and subsequent AII administration on SBP before and after baroreceptor stimulation, in the anaesthetised cat

The effect of captopril, and subsequent AII infusion, on resting SBP and the reflex pressor response to baroreceptor stimulation, was analysed in cats 1-3. The results are shown in table 1.11.

The results show that, as in the dog, captopril decreased SBP and, to a lesser extent, the reflex pressor response to baroreceptor stimulation. AII did not increase SBP above control values.

Comments

These results show that as expected, captopril lowers SBP. The hypotensive effect measured in these experiments is probably not the maximum hypotensive effect that captopril would normally produce. This is because we always started a continuous infusion of dextran, as described in the "methods" section, at the same time captopril was administered. This prevented any severe falls in SBP, as this would reduce the survival time of the anaesthetised preparation.

The results show that the same concentration of AII which restored both resting and reflex catecholamine release, after captopril administration, did not increase either resting SBP or the reflex pressor response. This supports our hypothesis that it is a

Table 1.11

The effect of captopril on resting systemic blood pressure (SBP) and the reflex increase in SBP following baroreceptor stimulation (BRS), in cats 1-3.

Cat number	Resting SBP			SBP following BRS			Increment		
	Control	Captopril	AII	Control	Captopril	AII	Control	Captopril	AII
1	70,70	50,50	65,70	120,95	70,70	100,110	50,25	20,20	35,40
2	55	50	80	90	60	80	35	10	20
3	100,70	80,90	100,100	120,110	135,130	140,130	20,40	55,40	40,30
Mean ± SE (n=5)	73.0 ± 7.3	64.0 ± 8.7	77.0 ± 10.0	107.0 ± 6.2	93.0 ± 16.2	112.0 ± 10.7	34.0 ± 5.3	29.0 ± 8.1	33.0 ± 3.7

minimum, non pressor, level of circulating AII which is required for the adrenal gland to respond to the reflex stimuli.

Feuerstein etal (1977) suggested that, after baroreceptor stimulation, the main site of action of AII was the central nervous system. Presumably the increase in sympathetic drive induced by AII would affect the vasculature and the adrenal medulla. If so, captopril would be expected to have a comparable inhibitory effect on the reflex pressor response and reflex adrenal catecholamine release induced by baroreceptor stimulation. From tables 1.1 and 1.2, it can be seen that captopril inhibits reflex catecholamine release by approximately 72%. From table 1.10, it can be seen that captopril inhibits the reflex pressor response by approximately 27%. So captopril has a greater inhibitory effect on adrenal catecholamine release. This indirectly supports our hypothesis that AII is exerting a permissive effect at the level of the adrenal gland rather than centrally.

5. The effect of captopril, followed by a continuous infusion of AII, on adrenal venous blood flow

During the experiments performed, after captopril administration, we observed an increase in adrenal blood flow.

To investigate the effect of captopril on adrenal venous blood flow, the effects of captopril and the subsequent administration of AII on adrenal blood flow in dogs 1-9 was analysed. The results are shown in table 1.12.

Table 1.12

Adrenal blood flow in dogs 1-9 (D1-9), before and after baroreceptor stimulation BRS), before and after captopril administration and a subsequent infusion of angiotensin II.

Drug Treatment	Time after onset of BRS (mins)	Adrenal blood flow ($ml min^{-1}$) D1	D2	D3	D4	D5	D6	D7	D8	D9	Mean ± SE
Control	0	4.1,4.1	0.8,0.5	3.7,3.2	2.3	2.9,2.9	2.5	3.8,4.0	2.1,1.9	3.3	2.8 ± 0.3 (n=15)
	1-2	5.0,4.4	2.8,1.7	4.9,5.2	4.8	3.7,3.6	5.3	6.1,6.5	3.8,3.0	4.9	4.4 ± 0.3 (n=15)***
	5-6	4.7,4.8	1.6,0.8	4.8,3.8	3.5	3.8,3.5	4.9	5.5,5.7	6.2,3.4	2.9	4.0 ± 0.4 (n=15)***
	9-10	4.9,4.6	1.1,2.0	3.8,3.0	2.9	3.8,3.0	4.1	5.5,5.7	3.0,3.1	3.0	3.6 ± 0.3 (n=15)***
Captopril (25mg)	0	5.8,6.2	1.9,1.0	4.0,2.8	3.5,2.8	2.7	4.2,4.1	6.0,4.8	1.5,3.0	2.9,2.7	3.5 ± 0.4 (n=17)**
	1-2	6.2,6.5	2.2,1.5	7.0,3.2	5.8,6.0	3.2	7.2,6.0	8.0,7.2	4.2,3.5	8.8,7.1	5.5 ± 0.5 (n=17)***
	5-6	5.9,6.0	1.9,0.6	2.3,3.0	4.5,4.2	3.1	6.0,2.0	7.4,6.1	3.2,2.8	5.3,5.2	4.1 ± 0.5 (n=17)***
	9-10	5.5,5.7	1.5,0.7	2.7,2.4	4.0,2.8	2.9	4.8,2.0	6.8,5.5	2.5,4.3	5.8,5.6	3.9 ± 0.4 (n=17)***
AII ($1-5 ng min^{-1}$)	0	4.1	1.0,1.9	1.2,3.0	2.0,1.5	1.9,1.5					1.9 ± 0.4 (n=9)*
	1-2	4.8	0.7,1.8	6.0,4.0	3.8,1.0	2.5,2.1					3.0 ± 0.6 (n=9)*
	5-6	4.7	0.6,3.0	4.5,3.5	2.8,0.8	2.3,2.6					2.8 ± 0.5 (n=9)*
	9-10	4.7	1.6,2.4	4.4,2.6	2.3,0.7	2.2,2.9					2.6 ± 0.4 (n=9)*

Statistical significance (data before BRS compared with data after BRS, captopril data before BRS compared with control data before BRS and AII data before BRS compared with captopril data before BRS)-:

* - $p > 0.02$
** - $p > 0.01$
*** - $p > 0.001$

The results show that there was a significant increase in adrenal blood flow during baroreceptor stimulation both before and after captopril and AII administration. Captopril induced a significant increase in resting blood flow, prior to baroreceptor stimulation. AII significantly decreased resting adrenal blood flow, reversing the effect of captopril.

6. The effect of resting systemic blood pressure on resting adrenal venous blood flow

The observation that captopril induced an increase in adrenal blood flow despite its hypotensive effect was surprising as we expected a decrease in blood flow to occur with a decrease in SBP.

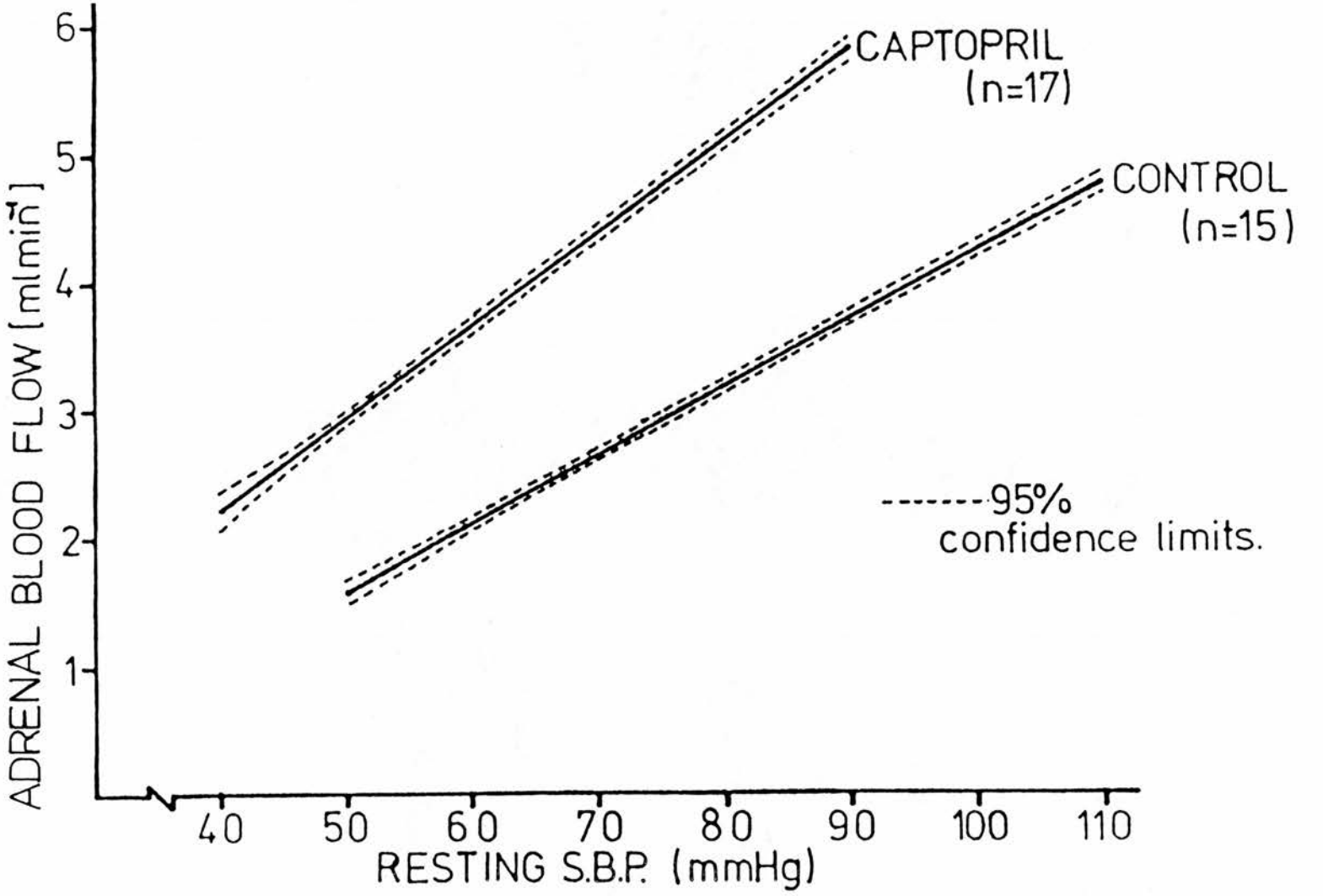
To investigate this, a resting systemic blood pressure - blood flow graph was constructed for dogs 1-9. The linear regression lines with 95% confidence limits are shown in figure 1.8. The data used to compile this figure can be found in tables 1.10 and 1.12.

The graph shows that there is a positive relationship between adrenal blood flow and resting SBP. This would account for the significant increase in adrenal venous blood flow following baroreceptor stimulation induced pressor responses.

Captopril produced a significant elevation of the linear regression line, indicating that AII may have an inhibitory role in the maintainance of adrenal blood flow. An alternative explanation is that the blood becomes diluted due to the continuous infusion of

Figure 1·8

Linear regression lines for resting systemic blood pressure (SBP) vs. adrenal blood flow before and after captopril administration



dextran administered after captopril administration. Blood flow is inversely proportional to the viscosity of the blood, and table 1.13 was constructed to investigate if there was a reduction in haematocrit after captopril administration.

Table 1.13 shows that there was a significant reduction in haematocrit following captopril administration, probably due to dilution of the blood by dextran. It seems possible that some of the increase in adrenal blood flow after captopril administration was due to the reduction in adrenal venous blood haematocrit. The dextran infusion was usually slowed down or stopped after AII infusion. The reduction in adrenal blood flow following AII may be due to this decrease in dextran infusion. A real effect of captopril on adrenal blood flow, however, cannot be ruled out.

7. The effect of adrenal venous blood flow on catecholamine release

In order to exclude the possibility that the increase in adrenal catecholamine release following baroreceptor stimulation was due to the simultaneous increase in adrenal blood flow, a catecholamine release - blood flow graph was constructed for dogs 1-9 (for control values only). The linear regression line, with 95% confidence limits is shown in figure 1.9. The figure was compiled from data contained in tables 1.1 and 1.12.

The graph shows that the total catecholamine release is independant of blood flow. The increased reflex release of catecholamines, after baroreceptor stimulation is therefore not due to

Table 1.13

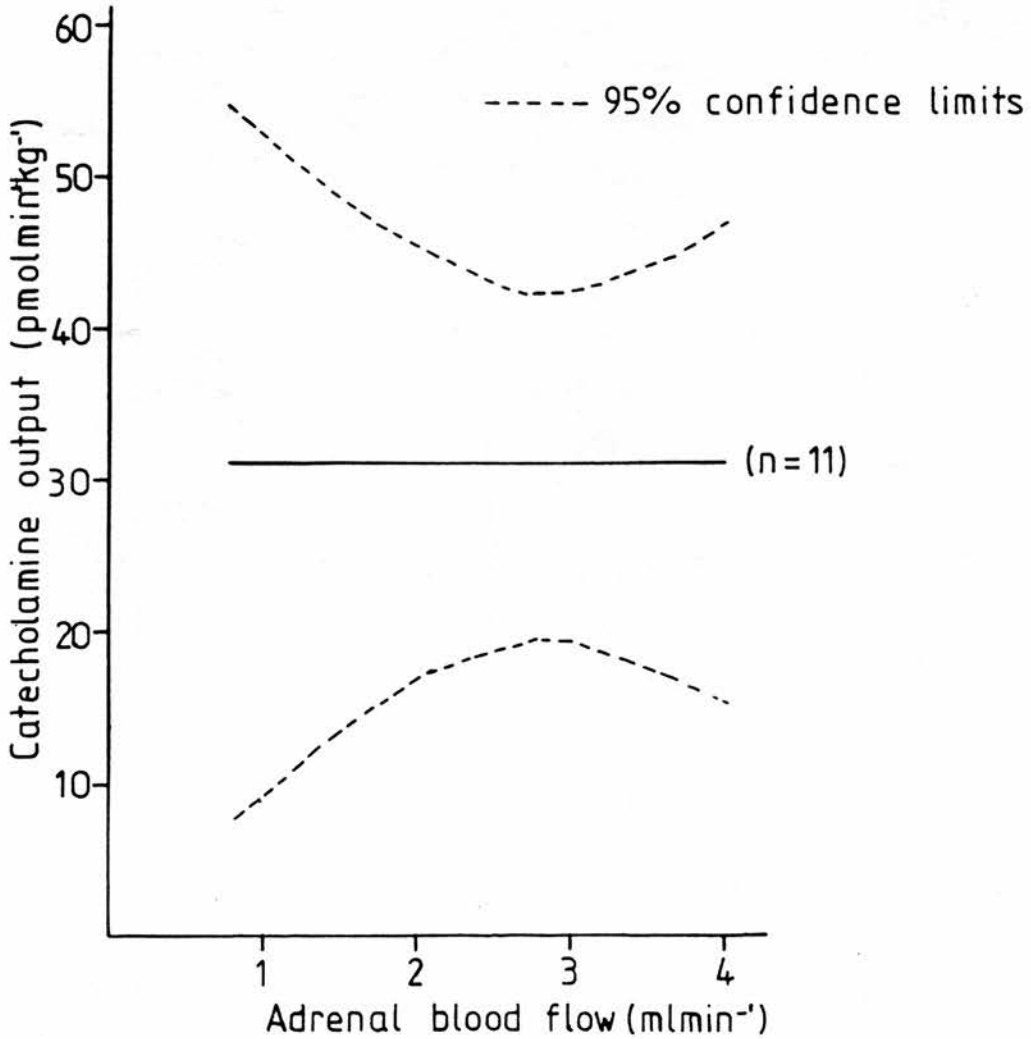
The effect of captopril administration on adrenal venous blood haematocrit in dogs 1-9.

Dog number	Haematocrit before captopril	Haematocrit after captopril
1	59,61	47,32
2	50,40	37,30
3	54,53	35,32
4	61	31,29
5	52,55	33
6	48	38,37
7	53,50	42,38
8	52,47	33,30
9	55	41,41
Mean ± SE	(n=15) 52.7 ± 1.4	(n=17) 35.6± 1.2 *

Statistical significance (compared with pre-captopril data)-:
* = $p < 0.001$

Figure 1·9

Linear regression line for adrenal blood flow vs catecholamine output (pre-captopril control values only) for dogs 1-9.



the simultaneous increase in adrenal blood flow.

8. The effect of resting systemic blood pressure on resting adrenal catecholamine output before and after captopril administration

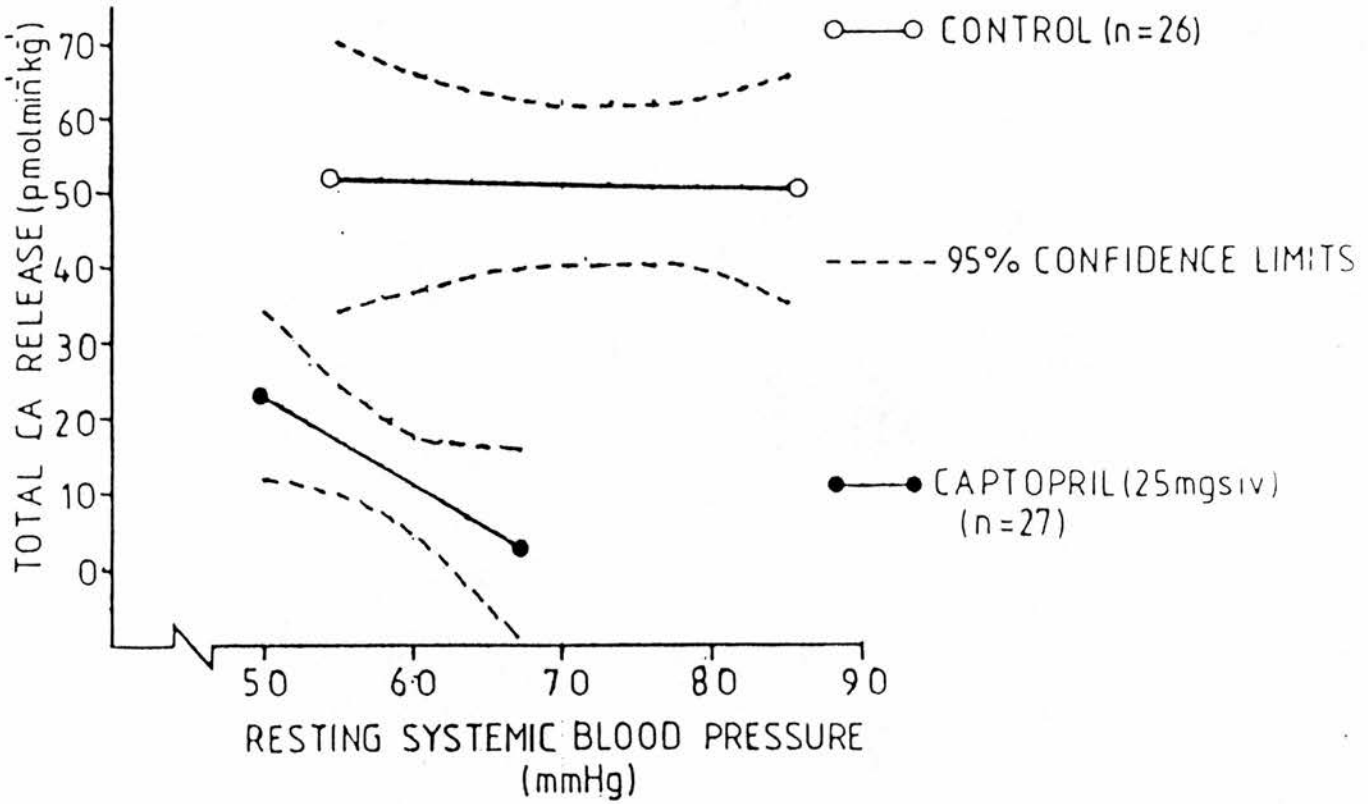
In order to exclude the possibility that the reduction in adrenal catecholamine release following captopril administration was due to the decrease in SBP induced by captopril, a resting SBP - catecholamine release graph was constructed. The linear regression lines with 95% confidence limits are shown in figure 1.10. The figure was compiled from data contained in tables 1.1 and 1.10.

The graph shows that total catecholamine release is independant of the resting SBP. Captopril produced a significant depression of the linear regression line. After captopril, although not significant, a decrease in SBP produced a tendency towards an increase in total catecholamine release. These results exclude the possibility that the inhibition of adrenal catecholamine release induced by captopril was due to the simultaneous reduction in SBP.

9. The effect of resting systemic blood pressure on the reflex increase in systemic blood pressure following baroreceptor stimulation

It was of interest to investigate if the resting SBP had an effect on the reflex pressor response induced by baroreceptor stimulation. If a reduction in resting SBP reduced the reflex pressor

Figure 1·10



Linear regression lines for resting systemic blood pressure vs. total catecholamine (CA) release before and after captopril administration

response, this could explain the reduction in the reflex pressor response following captopril administration.

To investigate this a resting SBP - pressor response graph was constructed using the control values contained in table 1.10. The linear regression line with 95% confidence limits is shown in figure 1.11.

The figure shows that there is not a significant relationship between resting SBP and the reflex pressor response. There is, although not significant, a tendency towards a positive relationship between resting SBP and the reflex pressor response. The reduction in SBP induced by captopril could contribute to its reduction of the reflex pressor response.

10. The effect of captopril on the resistance of the vascular bed of the hind limb in the anaesthetised dog

As will be reviewed and discussed in detail in "Part 3" of this thesis, there is much evidence to suggest that AII exerts a facilitatory effect on stimulation-induced noradrenaline release from most sympathetic nerve endings. AII has a similar effect on the release of acetylcholine from both sympathetic and cholinergic ganglia. From Tables 1.1 and 1.2 it can be calculated that captopril inhibits resting catecholamine release and the change in catecholamine release following baroreceptor stimulation by approximately 30% and 72% respectively. From table 1.10 it can be calculated that captopril inhibits resting SBP and the reflex pressor response to baroreceptor

Linear regression line for resting systemic blood pressure (S.B.P.) vs. the pressor response to baroreceptor (BR) stimulation (control data)

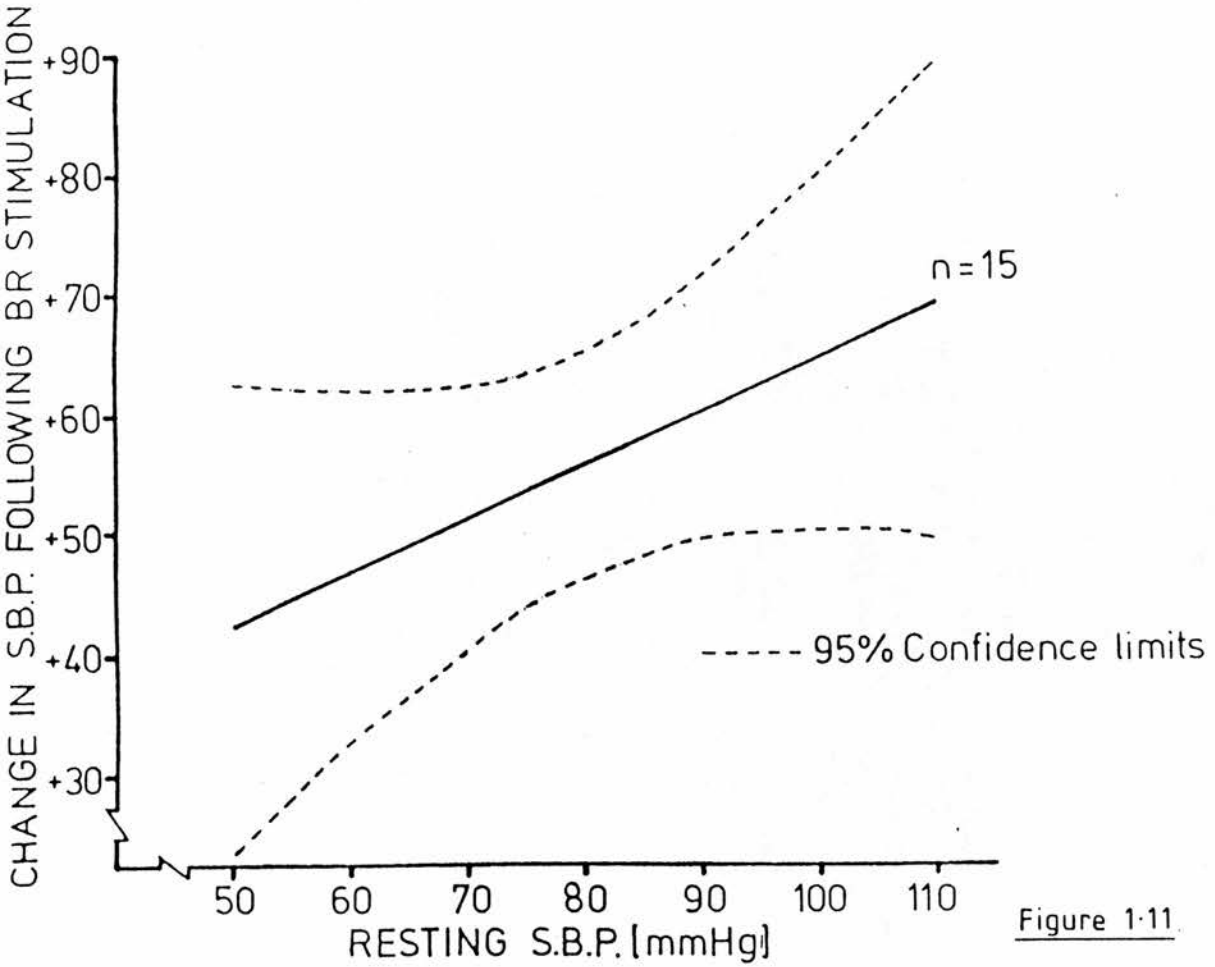


Figure 1·11

stimulation by approximately 19% and 27% respectively. It is evident that captopril inhibits the blood pressor responses less than the release of catecholamines. It was of interest to investigate the effect of captopril on the vascular responses of the hind limb, which is adrenergic.

In four dogs, the reflex SBP and hind limb perfusion pressure (HLPP) responses to baroreceptor stimulation were studied, before and after captopril administration. As flow through the perfused limb is constant, the perfusion pressure was taken as a measure of vascular resistance (see "Methods - Whole animal experiments"). The results are shown in tables 1.14 and 1.15.

The results show that, as has been observed, captopril significantly inhibits the reflex pressor response to baroreceptor stimulation. Captopril did not, however, significantly affect the reflex increase in hind limb perfusion pressure. These results indicate that the renin - AII system has varying influences on reflex vascular resistance changes in different vascular beds.

Table 1.14

The effect of captopril on systemic blood pressure and hind limb perfusion pressure following baroreceptor stimulation.

	CONTROL			CAPTOPRIL		
	CPP	SBP	HLPP	CPP	SBP	HLPP
DOG A	140→100	90→130	100→140	140→100	60→70	90→100
	"	70→100	120→160	"	60→70	95→110
	"	50→90	120→200	"	60→70	100→130
	"	45→65	100→140			
DOG B	140→100	110→165	110→150	140→100	120→150	110→150
	"	130→175	120→160	"	100→130	120→160
				"	100→140	120→165
				"	100→130	100→150
DOG C	140→100	110→170	110→160	130→90	90→155	110→170
	"	100→170	100→155	120→80	80→120	100→150
	130→90	90→175	110→160			
	140→100	115→180	115→150			
DOG D	130→90	130→180	100→130	120→80	95→145	100→145
	"	140→180	100→130	130→90	110→160	110→145
Mean ± SE	137.5±1.3	98.3±8.9	108.8±2.5	134.5±2.5	88.6±6.3	105.0±2.9
	→	→	→	→	→	→
	97.5±1.3	148.3±11.9	152.9±5.4	94.5±2.5	121.8±10.6	143.2±6.6

CPP = carotid perfusion pressure
SBP = systemic blood pressure
HLPP = hind limb perfusion pressure

Table 1.15

The effect of captopril on the change in systemic blood pressure and hind limb perfusion pressure induced by baroreceptor stimulation.

	CONTROL			CAPTOPRIL		
	CPP	SBP	HLPP	CPP	SBP	HLPP
DOG A	-40	+40	+40	-40	+10	+10
	"	+30	+40	"	+10	+15
	"	+40	+8	"	+10	+20
	"	+20	+40	"	+30	+50
DOG B	-40	+55	+40	-40	+30	+40
	"	+45	+40	"	+30	+40
				"	+40	+45
				"	+30	+50
DOG C	-40	+60	+50	-40	+65	+60
	"	+70	+55	"	+40	+50
	"	+85	+50			
	"	+65	+35			
DOG D	-40	+50	+30	-40	+55	+45
	"	+40	+30	"	+40	+35
Mean ± SE	-40.0±0	+50.0±5.2	+44.2±3.9	-40.0±0	+32.7±5.5 *	+37.5±4.8

CPP = carotid perfusion pressure
SBP = systemic blood pressure
HLPP = hind limb perfusion pressure

Statistical significance (captopril data compared with control data)-:
* = $p < 0.01$

Part 1 - Summary of results

1. Captopril reduced both the resting release of catecholamines from the adrenal medulla, and the reflex release of catecholamines induced by baroreceptor stimulation, in anaesthetised dogs and cats (25mg and 5mg of captopril respectively).

2. Plasma renin activity did not increase during the ten-minute baroreceptor tests.

3. An exogenous infusion of angiotensin II (AII) ($1-5 ng min^{-1}$) partially reversed the inhibitory effect of captopril on resting and reflex adrenal catecholamine release in the anaesthetised dog. The reversal was more complete in the anaesthetised cat.

4. Captopril reduced both resting systemic blood pressure and the reflex pressor response to baroreceptor stimulation in anaesthetised dogs and cats.

5. The level of AII which reversed the effects of captopril on adrenal catecholamine release had no effect on resting systemic blood pressure or the reflex pressor response to baroreceptor stimulation.

The following points refer to anaesthetised dogs only -:

6. Captopril increased adrenal blood flow. Dilution of the blood by dextran may contribute to this effect. AII reversed the effect of captopril on adrenal blood flow. Baroreceptor stimulation increased

adrenal blood flow.

7. The increase in adrenal blood flow during baroreceptor stimulation may be explained by the reflex increase in systemic blood pressure, as there was a positive relationship between resting systemic blood pressure and adrenal blood flow.

8. Adrenal catecholamine release is independant of adrenal blood flow, and so the catecholamine release induced by baroreceptor stimulation was not a result of the simultaneous increase in adrenal blood flow.

9. Adrenal catecholamine release is independant of systemic blood pressure, so the reduction in adrenal catecholamine release after captopril administration was not a result of the simultaneous reduction in systemic blood pressure.

10. There is a positive relationship between resting systemic blood pressure and the reflex pressor response induced by baroreceptor stimulation. The reduction in resting systemic blood pressure by captopril may contribute to its reduction in the reflex pressor response to baroreceptor stimulation.

11. The renin-AII system has varying influences on reflex vascular resistance changes in the different vascular beds described.

Part 1 - Discussion of results

The results show that baroreceptor stimulation induced a reflex release of catecholamines from the adrenal medulla, as first demonstrated by Bedford and Jackson in 1916 and Heymans in 1929. These results support the findings of other workers, that haemorrhage induces a reflex release of catecholamines from the adrenal medulla (see "Introduction and literature review").

These results exclude the possibility that such a reflex release of catecholamines is the result of the simultaneous increase in adrenal blood flow that occurs, as it is shown that adrenal catecholamine release is independant of adrenal blood flow.

As was discussed in the "Introduction and literature review", activation of the renin-AII system has long been implicated in the response to hypovolemic and hypertensive states. Blockade of the renin-AII system has been shown to inhibit the adrenomedullary response to prolonged haemorrhage in cats and dogs (Harrison etal, 1973; Feuerstein etal, 1977). What had not been investigated was the role the renin-AII system plays in the acute response to such reflex stimuli. The results show that captopril inhibited the immediate release of catecholamines which occurs 1-2 minutes after the onset of baroreceptor stimulation. The possibility that this was due to the simultaneous hypotensive effect of captopril was ruled out, as it can be seen that adrenal catecholamine release is independant of resting systemic blood pressure. The effect of captopril on both the resting and reflex release of catecholamines was partially reversed, in the

dog, by the infusion of a non-pressor level of AII. The reversal was more complete in the cat. The experiments carried out on the cat were the first ones done, and hence a new batch of AII was used. It subsequently became clear that the activity of AII deteriorated over a period of time unless stored at a slightly acidic pH. It is possible that the activity of AII in the initial series of experiments carried out in dogs, that the activity of AII was less than that administered to the cats. This may explain why the AII was more effective at reversing the effects of captopril in the cats. In "Part 3" it will be shown that this level of AII was more effective at reversing the effect of captopril on splanchnic nerve stimulation-induced adrenal catecholamine release, and this may be due to the increased precautions taken when storing AII.

These results suggest that a minimum, non-pressor level of AII is required for the adrenal gland to respond to the reflex stimulus, and that AII has a direct facilitatory effect on both resting and reflex adrenal catecholamine release.

These conclusions are compatible with the findings and conclusions of Hatton, Clough, Adiguin and Conway (1982). They used lower body negative pressure to stimulate sympathetic reflexes in anaesthetised cats. This induced a transient decrease in systemic blood pressure (SBP). AII-converting enzyme inhibitors (ACEI's) and saralasin produced a greater, more sustained fall in SBP and this was not associated with a decrease in efferent nerve activity. This indicates that they were exerting their effects on the peripheral actions of AII. They concluded that AII, at a level which does not

exert a direct vasoconstriction, interacts with the sympathetic nervous system to maintain arterial pressure when homeostatic reflexes are activated, and that the reduction in the efficiency of these reflexes by ACEI's may contribute to their hypotensive effect.

My results support these conclusions, and suggest that a non-pressor level of AII also interacts with the sympathetic nervous system at the level of the adrenal gland.

Feuerstein etal (1977) attributed the adrenal response to haemorrhage in the cat to an activation of the renin-AII system, as they detected an increase in plasma renin activity (PRA) following haemorrhage. Studying their results, there appeared to be no significant increase in PRA up to ten minutes after haemorrhage, and they began their readings for PRA and adrenal catecholamine release at this time, but their results show a maximum release of catecholamines ten minutes after haemorrhage. Ten to twenty minutes after haemorrhage they demonstrated a significant rise in PRA.

My results show that there was no increase in PRA during the ten minute baroreceptor tests and there was an immediate reflex release of catecholamines 1-2 minutes after the onset of baroreceptor stimulation. They do confirm that the integrity of the renin-AII system is required for the adrenal response to baroreceptor stimulation, but not that the response is attributed to an activation of the renin-AII system. Furthermore, they suggest that a minimum, non-pressor level of AII is required for the adrenal gland to respond to the reflex stimulus, and that AII has a direct permissive effect on

both resting and reflex adrenal catecholamine release. Removal of this level of AII by captopril would prevent the transmission of the adrenal reflex and subsequent replacement of AII would restore the adrenal reflex. The result show that this is the case.

Feuerstein etal (1977) also concluded that, following haemorrhage, AII was acting centrally to increase sympathetic drive, and hence adrenal catecholamine release via activation of the splanchnic nerve. My results suggest that the effect is not a central one, but a direct effect at the level of the adrenal gland, a minimum level of AII being required for the normal response of the adrenal gland to sympathetic drive. This is supported by the work of Hatton etal (1982) who showed that the hypotensive effect of ACEI's and saralasin, in the anaesthetised cat, was not associated with a decrease in efferent nerve activity.

As described in the "Results" section, captopril inhibited adrenal catecholamine release by 30-70%, while inhibiting the reflex pressor response by only 20-30%. Additionally, this reduction in the reflex pressor response may be partially explained by the hypotensive effect of captopril, as the results presented in 1.9 demonstrate that there is a tendency for a reduction in resting SBP to reduce the reflex pressor response. These results indirectly support my conclusions that the effect of AII is at the level of the adrenal gland, as if the effect was a central activation of sympathetic drive the effect of captopril on the reflex pressor response and the reflex adrenal response would be expected to be comparable.

Corwin, Seaton, Hamaji and Harrison (1985) published evidence which they considered compatible with the theory of Feuerstein etal (1977), that it is a central activation of central drive that is responsible for the adrenal response to haemorrhage in the anaesthetised dog. Their conclusions were based on the observation that an i.c.v. injection of AII restored the adrenal response to haemorrhage in nephrectomised dogs. As was discussed in the "Introduction and literature review", there is little doubt that, even in the absence of nephrectomy, an i.c.v. injection of AII will induce an increase in adrenal catecholamine release, and this could result in a reversal of the effects of either nephrectomy or saralasin. Although Corwin etal demonstrated that an i.c.v. injection of AII will support adrenal catecholamine release following haemorrhage, these results do not confirm conclusively that this is the physiological response to haemorrhage.

It is evident that PRA does increase some 10-20 minutes after haemorrhage (Feuerstein etal, 1977), and it is possible that the renin-AII system is activated after prolonged baroreceptor stimulation and that this is necessary to maintain arterial pressure when baroreceptor stimulation is prolonged.

It is not possible, from the results presented here, to rule out the possibility that AII is acting centrally, but the results of a further series of experiments, described in "Part 3", do support the conclusions I have made here, that the facilitatory effect of AII is a direct one at the level of the adrenal gland.

The results show that captopril induces an increase in PRA. This is usually observed following captopril administration and is probably due to an interruption of the negative feedback loop by which AII stimulates renin release (Silberbauer etal, 1982). As will be discussed in "Part 5", captopril has been shown to stimulate prostaglandin (PG) synthesis and PGs, especially PGI_2, are potent stimulators of renin release (Frolich, 1980). This may contribute to the observed increase in PRA following captopril administration. What is more important is that, as in the control situation, there is no increase in PRA during baroreceptor stimulation, after captopril administration.

Insulin hypoglycaemia, hypoxia, hypercapnia, cold shock and muscular exercize all stimulate adrenal catecholamine release (Callingham, 1975). R. C. Torazi remarked in a panel discussion (see Br. J. Clin. Pharmac., 1982 14, 139s-140s) that captopril blunted the pressor response to dynamic exercise in patients. This effect could also be explained by captopril impairing the facilitatory effect of AII on adrenal catecholamine release, rendering the gland unable to respond normally to dynamic exersize.

Weinberger (1982) reported that captopril induced a marked decrease in plasma noradrenaline levels in hypertensive patients and suggested that this was related to the fact that AII induces release of noradrenaline from nerve endings (see "Part 3"). It is possible that the reduction in plasma noradrenaline was due to captoprils' effect on resting adrenal catecholamine release, as there is much evidence to suggest that AII has no physiological effect on resting

release of noradrenaline from nerve endings (see "Part 3 - Introduction and literature review).

Loute, Guffens, Waucquez, Firre, Legrand, Klels and Adam (1984) reported that captopril effectively lowered blood pressure to normal and reduced urinary secretion of noradrenaline in a patient suffering from phaeochromocytoma. Donker (1984) reported that in another case of hypertension due to phaeochromocytoma, saralasin infusion reduced blood pressure and urinary noradrenaline secretion. Both Loute etal (1984) and Donker (1984) were unable to explain these observations. They could be explained by the results presented here. It is possible that captopril and saralasin were exerting these effects by inhibiting the facilitatory effect of AII on the phaeochromocytoma affected chromaffin cells. It may be that such cells are more responsive to circulating AII.

Lilly, Engeland and Gann (1982), reported that, in the anaesthetised dog, the adrenal medullary response to haemorrhage was potentiated after a second small haemorrhage was induced. They suggested that there is a factor associated with haemorrhage that potentiates the adrenal medullary response to a second small haemorrhage. I observed throughout this research, that the second control baroreceptor test usually induced a greater release of adrenal catecholamine release (eg, see table 1.1). Table 1.1 also shows that this effect was not observed following captopril administration and so it is possible that AII may be the factor Lilly etal were describing.

ACEIs have also been shown to lower blood pressure, in patients,

more in an erect than in a recumbent position (Marganti, Pickering, Lopez-Ovejaro and Laragh, 1980). This could be due to a reduction in the normal reflex adrenal resonse to adopting an erect pose from a recumbant position, although I found little evidence to suggest that captopril induces postural hypotension, except perhaps in the aged (this was suggested to me in a conversation with a general practitioner).

The results show that there was a significant increase in adrenal blood flow during baroreceptor stimulation. The reflex pressor response could contribute to this effect as shown in figure 1.8, which shows that an increase in systemic blood pressure induces an increase in adrenal blood flow. This increase in adrenal blood flow following baroreceptor stimulation may be physiologically important, facilitating removal of the released catecholamines away from the adrenal gland, preventing accumulation of vasoactive catecholamines in the adrenal veins. It woud also provide additional oxygenation of the gland which would support its increase in metabolic activity.

Captopril was shown to increase adrenal blood flow. Table 1.13 shows that there was an decrease in adrenal blood haematocrit following captopril administration. Dilution of the blood by dextran could explain this effect and contribute significantly to the increase in adrenal blood flow observed after captopril administration. An infusion of dextran was always started at the same time as captopril administration to prevent any severe fall in blood pressure which would have limited the survival of the anaesthetised preparation. AII did apparently reverse this effect of captopril on adrenal blood flow

which may be due to the fact that the dextran infusion was usually slowed down or stopped after AII administration. It is possible, however, that captopril was itself inducing an increase in adrenal blood flow. As will be described in "Part 5", captopril may induce release of prostaglandins from the adrenal medulla and prostaglandins may play a role in maintaining adrenal blood flow. The possibility that captopril reduces adrenal venous resistance, which would facilitate an increase in adrenal blood flow, is also investigated in "Part 5".

In conclusion, the results suggest that the integrity of the renin-AII system is required for the adrenal gland to respond to baroreceptor stimulation in the anaesthetised dog and cat. A minimum, non-pressor level of AII may be required for the gland to respond to the reflex stimulus, and activation of the renin-AII system is not required for the immediate adrenal response. The effect of AII is likely to be a direct facilitatory one on adrenal catecholamine release at the level of the adrenal gland and not through central activation of sympathetic drive, although this possibility cannot be ruled out.

Part 2

We hypothesised that one possible mechanism by which captopril could reduce catecholamine output from the adrenal medulla was via an inhibition of adrenocorticosteroids (ACS) secretion.

This was suggested by work previously carried out in this laboratory by Critchley, Ellis, Henderson and Ungar (1982), which suggested a possible role for the pituitary-adrenocortical axis in the reflex release of catecholamines from the adrenal medulla of the dog.

Anichkov, Malyghina, Poskalenko and Ryzhenkov (1960) and Marotta (1972) had previously shown that carotid chemoreceptor stimulation causes a release of ACTH from the anterior lobe of the pituitary gland, resulting in a release of ACS. Critchley etal (1982) noted that release of catecholamines from the adrenal medulla in response to carotid body hypoxia outlasted the stimulus. Adrenal denervation abolished the immediate release of catecholamines, whilst the prolonged release was abolished by cycloheximide, a drug that inhibits release of ACS in response to adrenocorticotrophic hormone (ACTH) (Garren, Ney and Davis, 1965). Cycloheximide did not affect the immediate release of catecholamines and denervation did not affect the prolonged release.

It had previously been shown that hydrocortisone could stimulate release of catecholamines from the isolated perfused adrenal gland in a dose-dependant manner and in concentrations normally detected in adrenal venous blood (Critchley, Henderson, Moffatt, Waite and West,

1975). ACTH had also been shown to induce a release of catecholamines from the adrenal medulla in the anaesthetised dog (Critchley and Ungar, 1974). Critchley etal showed that ACTH potentiated the release of catecholamines induced by baroreceptor stimulation, and the release of catecholamines followed a similar time course to that shown by prolonged chemoreceptor stimulation. They concluded that a component of the reflex adrenal catecholamine release is mediated by ACTH and ACS secretion.

It has been known for some time that corticosteroids induce the action of phenylethanolamine N-methyl transferase (PNMT) which converts noradrenaline to adrenaline in mammalian chromaffin cells (Wurtman, Pohorecky and Baliga, 1972). Wurtman, Caspar, Pohorecky and Bartter (1968) studied the effects of hypophysectomy and ACTH on the adrenal release of catecholamines, in response to insulin-hypoglycaemia, in the dog. They reported that hypophysectomy reduced PNMT activity and also reduced both the resting and reflex release of adrenaline. Release could be restored by administration of ACTH. They concluded that these results could be explained by the induction of PNMT by ACS. Critchley etal (1982) showed that the results of Wurtman etal (1968) could be recalculated to show that ACTH affected the release of noradrenaline as well as adrenaline. They suggested that induction of PNMT by ACS could be secondary to an effect on the release of catecholamines.

There is much evidence to support the suggestion that ACS affect the release of catecholamines from the adrenal medulla via an interaction with the renin-angiotensin (AII) system. There is also

much evidence to suggest an interaction of the renin-AII system with the activity of ACS. Cortisol may also play a role in the restoration of blood volume after haemorrhage (Ganten, Unger, Rockhold, Schaz and Speck,1979). Lilly, Engeland and Gann (1982) demonstrated that haemorrhage induced a release of adrenal cortisol and catecholamines in anaesthetised dogs.

Intracerebroventricular (icv.) injection of AII into conscious rats has been shown to induce an increase in plasma and adrenal corticosterone. This effect is mediated by ACTH as hypophysectomy abolishes the response (Daniels-Severs, Ogden and Vernikos-Danellis, 1971). An icv. infusion of AII in conscious dogs also causes a significant increase in plasma ACTH (Ramsey, Keil, Sharpe and Shinsako, 1978) and there is evidence that AII may stimulate ACTH release by a direct action on the anterior pituitary (Maran and Yates, 1977). An icv. injection of renin has also been shown to result in an increase in plasma catecholamines and corticosterone, in both normal and sodium-depleted dogs. This effect can be blocked by icv. injection of captopril (Scholkens, Jung, Rascher, Shomig and Ganten, 1980). This increase in plasma corticosteroid levels, induced by renin, is probably mediated by ACTH (Reid and Day, 1977).

Corticosterone has also been shown to increase brain angiotensinogen (Wallis and Printz, 1980) which would increase AII biosynthesis, as brain AII appears to stimulate an increase in plasma catecholamine and corticosterone levels, I suggest these interactions would provide a self-potentiating mechanism by which the renin-AII system and the pituitary-adrenocorticotrophic axis increase blood

pressure and maintain the circulating blood volume.

It is still not clear if AII stimulates cortisol biosynthesis. Early studies using dispersed human adrenal cells or in vivo infusions, were unable to show a significant stimulation of cortisol by AII (Ames, Borkowski and Sicinski, 1976; Williams and Braley, 1977). Douglas, Brown and White (1984) did demonstrate that binding of AII to normal human fasciculata cells and cortisol producing adenoma cells was accompanied by corresponding changes in cortisol production. Specific binding of AII to adrenal receptor sites has been demonstrated in the bovine, rat and dog adrenal cortex (Lin and Goodfriend, 1970; Glossman, Baukel and Catt, 1974; Douglas, Aguilera, Kondo and Catt, 1978). AII has been shown to stimulate cortisol secretion in bovine and dog adrenals (Carpenter, Davis and Ayres, 1961; Kaplan and Bartter, 1962) suggesting that there may be some species specificity (this will be discussed further in the "Discussion" section for "Part 2").

AII may potentiate the action of ACTH on the adrenal cortex. Slater, Barbour, Henderson, Casper and Bartter (1963) and Bravo, Khosla and Bumpus (1975) demonstrated that, in the dog, AII potentiates the response of ACTH on cortisol secretion. Fraser, Buckingham, Mason and Semple (1978) were unable to stimulate cortisol production by AII infusions alone in dexamethasone supressed normal volunteers, but when the AII was infused over a baseline of ACTH stimulation, cortisol secretion was potentiated. Parker, Lifrak, Kawahara, Geduld and Kozbur (1983), using dog adrenal cell suspensions, observed only a minimal cortisol secretion when AII was

infused alone, but demonstrated that AII potentiated ACTH induced cortisol secretion. Both AII and ACTH were infused at physiological concentrations and they suggested that this may explain the increase in adrenal androgens seen in some pathological conditions characterised by an increase in plasma renin activity. Morera, Andoka and Chauvin (1984) have also demonstrated that AII potentiates ACTH-induced cyclic AMP production in bovine cortico-adrenal cells in primary culture, and that saralasin inhibits this effect.

Marotta (1972) demonstrated that, in dogs, 11-hydroxycorticosterone in adrenolumbar blood increased within one minute after infusion of ACTH or induced hypoxia. He suggested that this was due to central stimulation of ACTH acting on the adrenal cortex but it is possible, considering the very short time involved and the evidence already discussed, that the release induced by hypoxia was due to activation of the renin-AII system, AII affecting ACS release directly.

As mentioned previously, the pituitary-adrenocortical axis also affects the renin-AII system although, unlike AIIs potentiating effect on this axis, the axis appears to exert mainly an inhibitory effect on the renin-AII system.

Andoka, Chauvin, Marie, Saez and Morera (1984) demonstrated that ACTH decreases the number of AII binding sites in bovine adrenal glomerular cells, an effect which could be mediated by cyclic AMP. Aguileri, Fujita and Catt (1981) presented evidence that ACTH produced a decrease in plasma renin activity which appeared to correlate with

the number of AII receptors on glomerulosa cells. They postulated that the decrease in AII receptors was secondary to the suppression of the renin-AII system but Andoka etal (1984) reinterpretated this as a direct action of ACTH on AII receptors in the adrenal cortex. Both explanations are possible as in vitro binding studies have shown that when AII levels are elevated, the number of AII receptors on the adrenal cortex increase (Aguilera, Hauger and Catt, 1978; Devyrick etal, 1978).

These inhibitory effects of ACTH on the renin-AII system would appear not to support a facilitation of AII stimulated catecholamine secretion from the adrenal medulla. They may demonstrate a complex negative feedback system with the pituitary-adrenocortical axis dampening the already proposed self-potentiating mechanism by which the renin-AII system and this axis interact to maintain or increase blood pressure.

The adrenocortical system does not exert entirely inhibitory effects on the renin-AII system however. As previously mentioned, corticosterone has been shown to increase brain angiotensinogen (Wallis and Printz, 1980) which would result in an increase of brain AII. Corticotrophin releasing factor (CRF) is anatomically distributed in brain regions suspected of participating in the regulation of the autonomic nervous system (Vale, Rivier, Brown, Spiess, Koob, Swansson, Bilezikjian, Bloom and Rivier, 1983) and CRF can act centrally, in the rat, to increase adrenal catecholamine secretion (Brown and Fisher, 1984), increasing noradrenaline more than adrenaline, an effect similar to that seen following cold exposure,

tail suspension and ether exposure in the rat.

Corticosterone has been shown to increase the sensitivity of vascular smooth muscle to vasopressor agents such as AII (Dietz, Schomig, Haebara, Mann, Rascher, Luth, Grunherz and Ross, 1978).

It would appear that there is a complex interaction between the renin-AII system and the adrenocortical system. One possible explanation combining the evidence discussed here could be that in times of stress, eg. cold exposure, haemorrhage etc., both systems are activated in a self-potentiating manner to restore eg. blood volume. After this, perhaps circulating AII and corticosteroids reach a critical level at which the adrenocortical system begins to dampen or "apply the brakes" on the potentiating effects of the renin-AII system, until "homeostasis" is restored.

The aim of this series of experiments was to investigate a possible interaction between the pituitary-adrenocortical axis and the action of AII on baroreceptor stimulation-evoked adrenal catecholamine release. As was discussed previously, haemorrhage has been shown to induce a release of both catecholamines and cortisol in dogs (Lilly etal, 1982), and this, together with the evidence of Critchley etal (1982), already discussed, suggests that AII may possibly induce adrenal release of catecholamines indirectly through facilitation of corticosteroid secretion and/or release. AII and corticosteroids may therefore interact to facilitate the reflex release of catecholamines from the adrenal medulla.

The aims of this series of experiments were to answer the following questions-:

1. Does cycloheximide, administered before and after captopril, affect the resting adrenal release of catecholamines and the reflex release induced by baroreceptor stimulation and the ability of AII to restore the adrenal response after captopril administration ?

2. Can the inhibition of resting and reflex adrenal catecholamine release, by captopril, be restored by the administration of ACTH ?

3. Does captopril affect cortisol or corticosterone secretion from the adrenal cortex ?

Part two - Results

1. The effect of cycloheximide on the release of catecholamines from the adrenal medulla, following captopril and subsequent AII administration

Cycloheximide has been shown to inhibit the release of adrenocorticosteroids (ACS) in response to adrenocorticotrophic hormone (ACTH) (Garren, Ney and Davis, 1965). It has also been shown to inhibit the release of catecholamines in response to ACTH (Critchley, Ellis, Henderson and Ungar, 1982). Critchley etal (1982) demonstrated that $50mgkg^{-1}$ cycloheximide reduced, though not significantly, the adrenal catecholamine response to baroreceptor stimulation.

It was of interest to investigate the effect of $50mgkg^{-1}$ cycloheximide on adrenal catecholamine release after captopril administration and during subsequent AII infusion. In one dog (dog 10) the effect of cycloheximide on the reflex adrenal catecholamine release, prior to captopril administration, was studied. This was done to verify the observations made by Critchley etal (1982).The results are shown in table 2.1.

The results show that cycloheximide increased the resting catecholamine release and inhibited, but did not abolish, the reflex release of catecholamines.

In dogs 2-5, $50mgkg^{-1}$ cycloheximide was administered after

Table 2.1

The effect of cycloheximide on catecholamine release in dog 10, before and after baroreceptor stimulation (BRS).

Drug Treatment	Time after onset of BRS (mins)	Total CA release	Increment from control	Mean CA release	Mean incrememt from control
Control	0	24,23		23.5	
	1-2	37,82	13,59	59.5	36
	5-6	68,82	14,59	75	36.5
	9-10	93	69	93	69
cycloheximide ($50mgkg^{-1}$)	0	46		46	
	1-2	80,16	19,15	48	17
	5-6	68,50	7,4	59	5.5
	9-10	66	20	49	20

Table 2.2

The effect of cycloheximide on total catecholamine release in dogs 2-5 before and after baroreceptor stimulation (BRS), following captopril administration and during an infusion of angiotensin II (AII).

Drug Treatment	Time after onset of BRS (mins)	Dog2	Dog3	Dog4	Dog5	Mean ± SE
Captopril, AII and cycloheximide ($50mgkg^{-1}$)	0	14	1	18	11	11.0 ± 3.6
	1-2	14	3	8	16	10.3 ± 3.0
	5-6	16	2	0	11	9.7 ± 4.1
	9-10	16	0	0	7	7.7 ± 4.6

(See table 1.7 for a comparison of these results with the results obtained prior to cycloheximide administration.)

captopril administration and during AII infusion. The effect of cycloheximide on resting release and the reflex release of catecholamines was analysed. The results are shown in table 2.2. These results can be directly compared with the results shown in table 1.7.

The results show that, in the presence of captopril and AII, cycloheximide inhibits resting adrenal catecholamine release and totally abolishes the reflex release of catecholamines induced by baroreceptor stimulation.

2. The effect of cycloheximide on resting systemic blood pressure and the reflex pressor response to baroreceptor stimulation

The effect of cycloheximide on the resting SBP and the reflex pressor response to baroreceptor stimulation was analysed in dogs 2-5. The results are shown in table 2.3. I have compared the effects of cycloheximide with those of AII in dogs 2-5. For a full comparison with the effects compared with control and post-captopril SBPs I refer the reader to table 1.10.

The results show that cycloheximide had no effect on either resting SBP or the reflex pressor response to baroreceptor stimulation.

Comment

These results indirectly support the hypothesis that, if there is an interaction between AII, ACS and catecholamine release, the

Table 2.3

The effect of (50mgkg^{-1}) cycloheximide (cyclo), in the presence of captopril and angiotensin II (AII) on resting systemic blood pressure (SBP) and the reflex pressor response to baroreceptor stimulation (BRS).

Dog number	Resting SBP		SBP following BRS		Increment	
	AII	cyclo	AII	Cyclo	AII	Cyclo
2	45,40	40	70,90	50	25,50	10
3	60,60	70	90,85	110	30,25	40
4	70,55	50	120,110	110	50,55	60
5	45,35	45	60,70	75	15,35	30
Mean	51.3 ± 4.2	51.3 ± 6.6	86.9 ± 7.3	86.3 ± 14.6	35.6 ± 5.1	35.0 ± 10.4

interaction is occuring at the level of the adrenal gland. If the interaction was at the level of the central nervous system, both the reflex release of catecholamines and the reflex pressor response to baroreceptor stimulation would be affected by cycloheximide. The results show that, after captopril and AII administration, cycloheximide inhibited the resting release of catecholamines and totally abolished the reflex release. It did not have any effect on resting SBP or the reflex pressor response.

3. The effect of cycloheximide on adrenal venous blood flow

ACTH has been shown to increase adrenal blood flow (e.g. Edwards, Hardy and Malinowska, 1975), so it was of interest to investigate the effect of cycloheximide on adrenal blood flow.

In dogs 2-5,the effect of cycloheximide on resting adrenal blood flow was analysed and compared with adrenal blood flow in the presence of captopril and AII (see table 1.12).

The resting adrenal blood flows ($ml min^{-1}$) following cycloheximide administration were as follows -:
Dog 2 = 2.1, dog 3 = 5.4, dog 4 = 1.2 and dog 5 = 2.0, mean = 2.7±0.9. This compares with a mean of 1.7±0.22 (n=8) for adrenal blood flow immediately prior to cycloheximide administration (see table 1.12).

The results show that there is no evidence for a significant effect of cycloheximide on adrenal blood flow.

4. The effect of ACTH on resting and reflex catecholamine release, following captopril administration

We had hypothesised that part of the permissive effect of AII on adrenal catecholamine release was an indirect one, through facilitation of ACS secretion. If this were so, ACTH would be expected to reverse the inhibitory effect of captopril on adrenal catecholamine release. To investigate this possibility, in dogs 7,8,9,11 and 12, 100µg ACTH was administered following captopril administration.

The effect of ACTH on resting catecholamine release before and after baroreceptor stimulation, and in the presence of captopril was analysed. The results are shown in table 2.4. (In dog 7, the samples obtained following ACTH administration were lost, but I have included the remaining results. Dog 7 was omitted in the statistical evaluation of the results.)

The effect of ACTH on the change in catecholamine release following baroreceptor stimulation, and in the presence of captopril was analysed. The results are shown in table 2.5. The results were combined and illustrated in figure 2.1.

The results show that ACTH had little effect on resting catecholamine release in these dogs, but restored the reflex release of catecholamines previously inhibited by captopril. Like AII, ACTH did not restore the reflex response completely.

Table 2.4

The effect of adrenocorticotrophichormone (ACTH) on the release of catecholamines, following captopril administration and before and after baroreceptor stimulation (BRS).

Drug Treatment	Time after onset of BRS (mins)	Dog11	Dog7	Dog8	Dog9	Dog12	Mean ± SE
Control	0	23	15,14	79	61	57	41.5 ± 11.3 (n=6)
	1-2		35,24	217	153	122	110.2 ± 36.4 (n=5)
	5-6	26	28,13	203	137	107	85.7 ± 31.1 (n=6)
	9-10	31	19,29	169	89	103	73.3 ± 23.8 (n=6)
Captopril (25mg)	0	49,68	10,14	67,48	24,21	48	38.8 ± 7.4 (n=9)
	1-2	31	19,14	58	40	73	39.2 ± 9.3 (n=6)
	5-6	22,39	15,14	98	28,55	63	41.8 ± 10.2 (n=8)
	9-10	27,74	13,16	63	29,57	96	46.9 ± 10.6 (n=8)
ACTH (100µg)	0	51,30		34,34	44,47	46,38	40.5 ± 2.7 (n=8)
	1-2	72,57		77,48	67,50	78,61	63.8 ± 4.1 (n=8)*
	5-6	37		62,67	85,67	88,66	67.4 ± 6.3 (n=7)*
	9-10	64,39		77,48	70,61	68,65	61.5 ± 4.4 (n=8)*

Statistical significance (ACTH data before BRS compared with ACTH data after BRS)-:

* = $p > 0.001$

Table 2.5

The effect of adrenocorticotrophichormone (ACTH) on the change in catecholamines release induced by baroreceptor stimulation (BRS)

Drug Treatment	Time after onset of BRS (mins)	Dog11	Dog7	Dog8	Dog9	Dog12	Mean ± SE
Control	0						
	1-2		20,10	139	137	65	+74.2 ± 27.6 (n=5)
	5-6	3	13,-1	125	121	50	+62.4 ± 25.9 (n=6)
	9-10	8	4,15	91	73	46	+39.5 ± 14.9 (n=6)
Captopril (25mg)	0						
	1-2	-18	9,0	10	16	25	+7.0 ± 6.0 (n=6)
	5-6	-27,-29	5,0	31	4,34	15	+4.1 ± 8.3 (n=8)**
	9-10	-22,6	3,2	15	5,36	48	+11.6 ± 7.7 (n=8)*
ACTH (100µg)	0						
	1-2	21,27		43,14	23,3	32,23	+23.3 ± 4.2 (n=8)*
	5-6	7		28,33	41,20	42,28	+28.4 ± 4.6 (n=7)*
	9-10	13,9		43,14	26,14	22,27	+21.0 ± 3.9 (n=8)

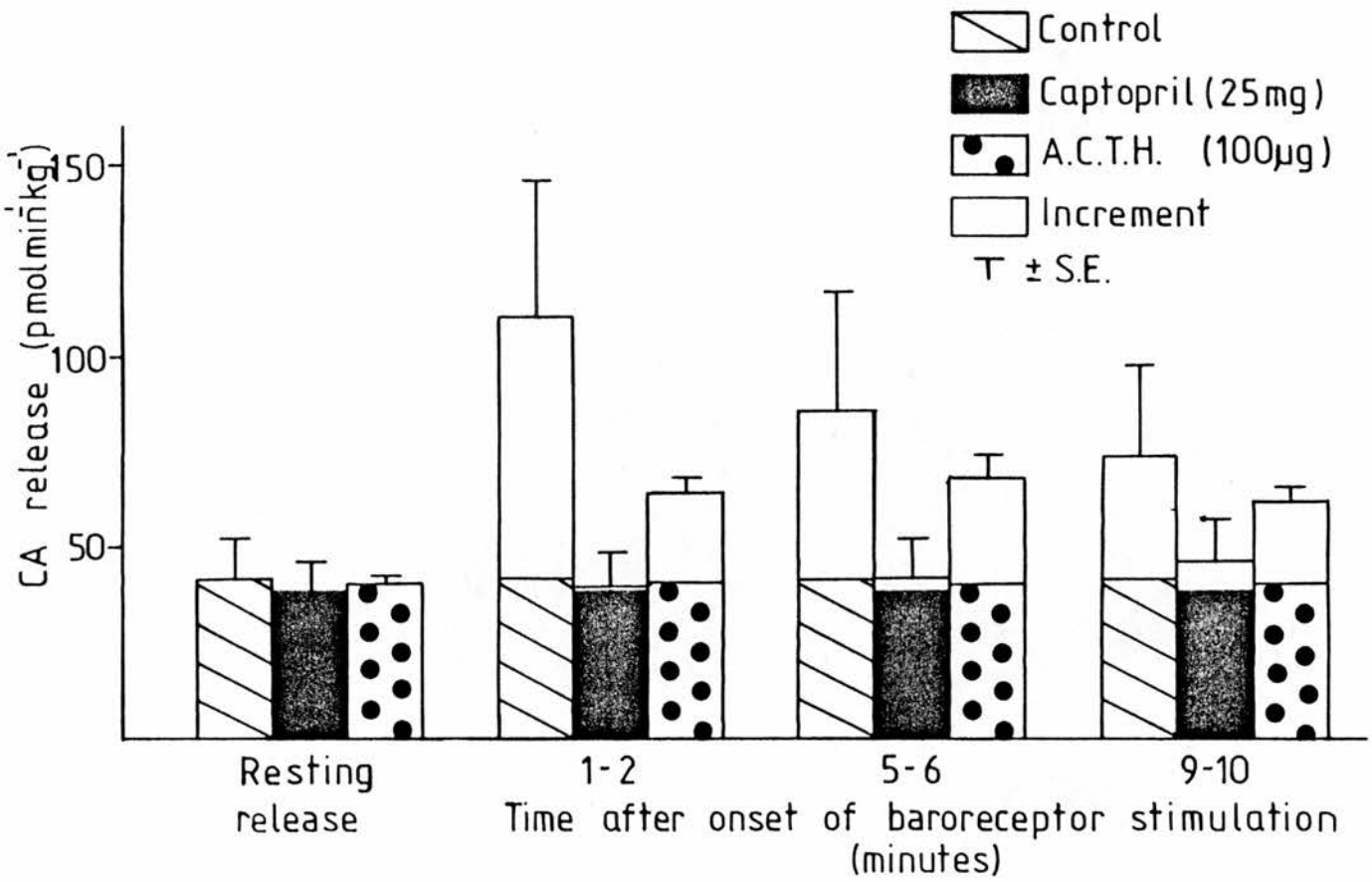
Statistical significance (captopril data compared with control data and ACTH data compared with captopril data)-:

* = $p < 0.05$

** = $p < 0.02$

Figure 2·1

The effect of adrenocorticotrophic hormone (A.C.T.H.), following captopril administration, on adrenal catecholamine (CA) release before and after baroreceptor stimulation.



5. The effect of ACTH on resting systemic blood pressure and the reflex response to baroreceptor stimulation

The effect of ACTH on resting systemic blood pressure (SBP) and the reflex pressor response to baroreceptor stimulation was analysed in dogs 7, 8, 9, 11 and 12. The results are shown in table 2.6.

Table 2.6 shows that, as has been previously demonstrated, captopril inhibits both the resting SBP, and the reflex pressor response to baroreceptor stimulation. There was no significant additional effect of the subsequent administration of ACTH on either resting SBP or the reflex pressor response.

Comments

ACTH is administered some six hours after the onset of anaesthesia. AII failed to restore SBP after captopril administration at this time point, in fact there was evidence of a further decrease in resting SBP and the reflex SBP response following AII administration (see table 1.10). As was discussed, this was probably not due to AII itself, but to the SBP already falling due to the length of time the preparation had been in use. If this is taken into consideration, then if ACTH was having no effect on SBP, both the resting SBP and the reflex pressor response would have been expected to fall after ACTH administration. The results show that there was in fact no change in either resting SBP or the reflex pressor response after ACTH administration. I suggest that ACTH may have increased SBP, but this increase was masked by the fall in SBP that usually occured

Table 2.6

The effect of adrenocorticotrophic hormone (ACTH), following captopril administration, on resting systemic blood pressure (SBP) before and after baroreceptor stimulation, and the change in SBP induced by baroreceptor stimulation (BRS).

Dog number	Resting SBP			SBP following BRS			Increment		
	Control	Captopril	ACTH	Control	Captopril	ACTH	Control	Captopril	ACTH
7	100,110	90,90	90,95	195,200	170,170	175,170	95,90	80,80	85,75
8	90,80	75,70	60,80	150,120	125,120	90,135	60,50	50,50	30,55
9	90	70,75	70,70	140	100,120	120,110	50	30,45	50,40
11	60	50,50	55,55	100	90,80	70,80	40	40,30	15,25
12	110	95	80,70	160	110	110,95	50	15	30,25
Mean ± SE	91.4 ± 6.7 (n=7)	**73.9 ± 5.5 (n=9)	72.5 ± 4.4 (n=10)	152.1 ± 13.9	120.6 ± 10.6	115.5 ± 11.2	62.1 ± 8.2	*46.7 ± 7.3	43.0 ± 7.3

Statistical significance (captopril data compared with control data)-:
* = $p > 0.02$
** = $p > 0.001$

around 6-7 hours after the start of each experiment. ACTH may have protected the preparation from this fall in blood pressure.

6. Adrenal cortisol and corticosterone output and plasma concentration before and after captopril administration, and after a subsequent infusion of AII

If, as we hypothesised, AII is exerting a permissive effect on adrenal catecholamine release through facilitating adrenocorticosteroid (ACS) secretion, captopril would be expected to decrease ACS secretion, and a subsequent infusion of AII expected to reverse the effect of captopril.

Corticosterone output ($\mu g min^{-1} kg^{-1}$) and concentration ($\mu g ml^{-1}$ plasma) were analysed in adrenal venous blood, before and after captopril administration, and after the subsequent infusion of AII, in dogs 13-18. The results are shown in table 2.7 and illustrated in figure 2.2. Cortisol output and concentration were analysed also. The results are shown in table 2.8 and illustrated in figure 2.3.

The results show that captopril had little effect on corticosterone output. It did, however, significantly decrease corticosterone concentration, although AII did not reverse this effect. Captopril increased cortisol output, although AII did not significantly reverse this effect. Captopril did not significantly decrease cortisol concentration although there was evidence of some decrease.

Table 2.7

Adrenal corticosterone concentration ($\mu g ml^{-1}$ plasma) and output ($\mu g min^{-1} kg^{-1}$) in dogs 13-18, before and after captopril administration, and after a subsequent infusion of angiotensin II (AII).

Dog number and drug treatment	Corticosterone concentration	Plasma volume per minute	Corticosterone output
Control			
13	2.94,2.16	2.8,2.8	0.33,0.24
14	4.59,5.31,4.31	1.1,1.0,1.7	0.28,0.30,0.39
15	2.44,2.36	0.8,1.2	0.08,0.11
16	2.71,1.75	2.4,3.6	0.26,0.25
17	2.74,1.72	2.5,2.5	0.33,0.20
18	3.67,3.68	1.3,0.8	0.16,0.10
Mean ± SE (n=13)	3.10 ± 0.31	1.88 ± 0.26	0.23 ± 0.30
Captopril			
13	1.96,2.14	4.6,4.6	0.36,0.39
14	2.10,2.87	3.9,2.4	0.46,0.38
15	1.09,0.84	1.9,1.6	0.08,0.05
16	1.77,2.29	3.3,2.8	0.3,0.2
17	2.25,1.81	2.5,1.7	0.27,0.15
18	3.41,2.74	1.5,1.8	0.20,0.14
Mean ± SE (n=12)	2.10 ± 0.21	2.72 ± 0.33 *	0.25 ± 0.04
AII ($5 ng min^{-1}$)			
13	3.44,4.06	2.4,2.0	0.33,0.32
14	2.66,1.83	2.0,3.7	0.30,0.38
15			
16	3.13,1.74	1.5,2.3	0.19,0.16
17	3.08,1.79	1.3,1.2	0.19,0.10
18	1.26,1.01	1.8,1.8	0.08,0.06
Mean ± SE (n=10)	2.4 ± 0.32	2.0 ± 0.23	0.21 ± 0.04

Statistical significance (captopril data compared with control data)-:
* = $p > 0.02$

Figure 2·2

The effect of captopril (25 mg) and angiotensin II (AII), in the presence of captopril, on the output of corticosterone (A.) and the concentration of corticosterone (B.) from the left adrenal gland of the anaesthetised dog.

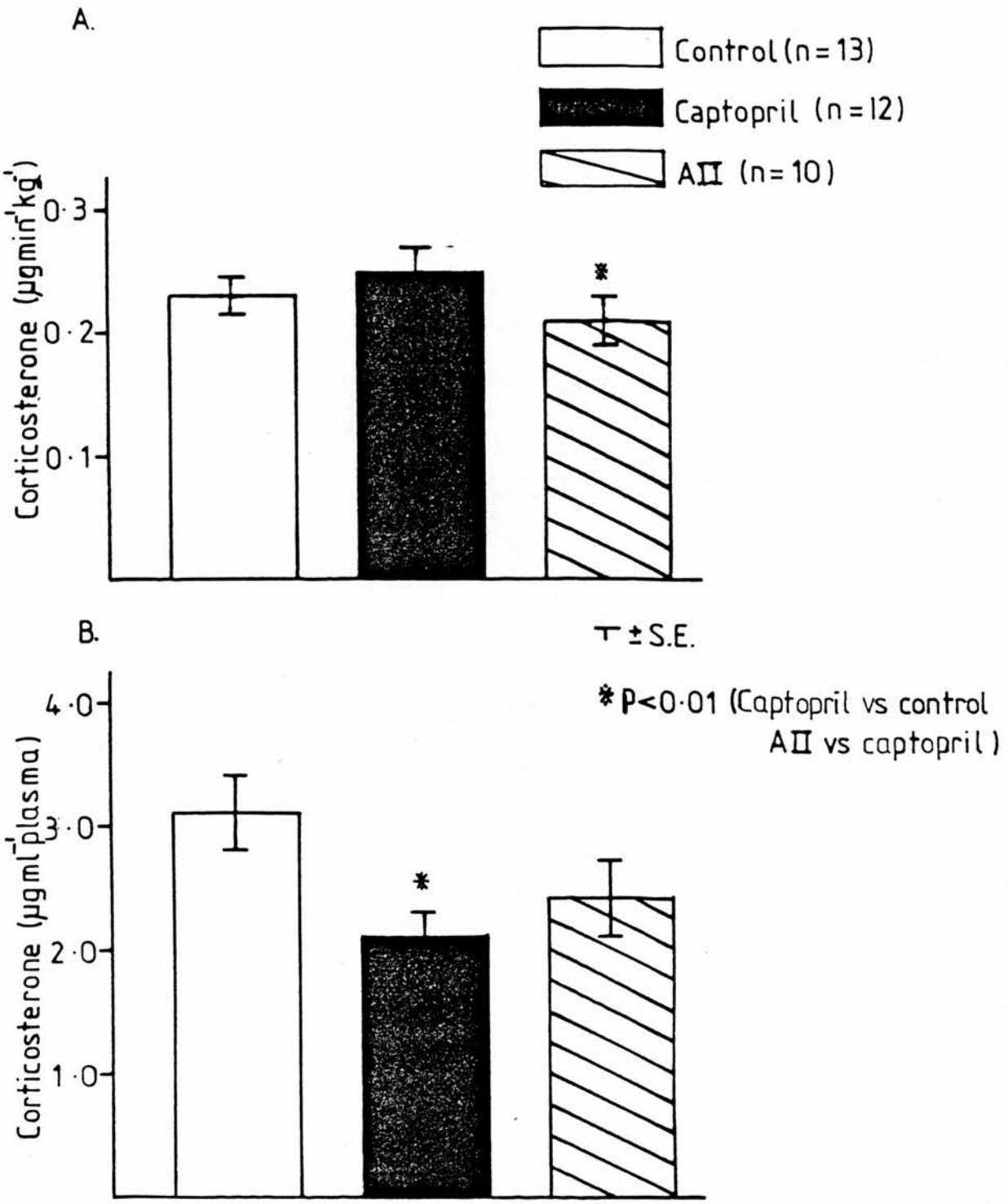


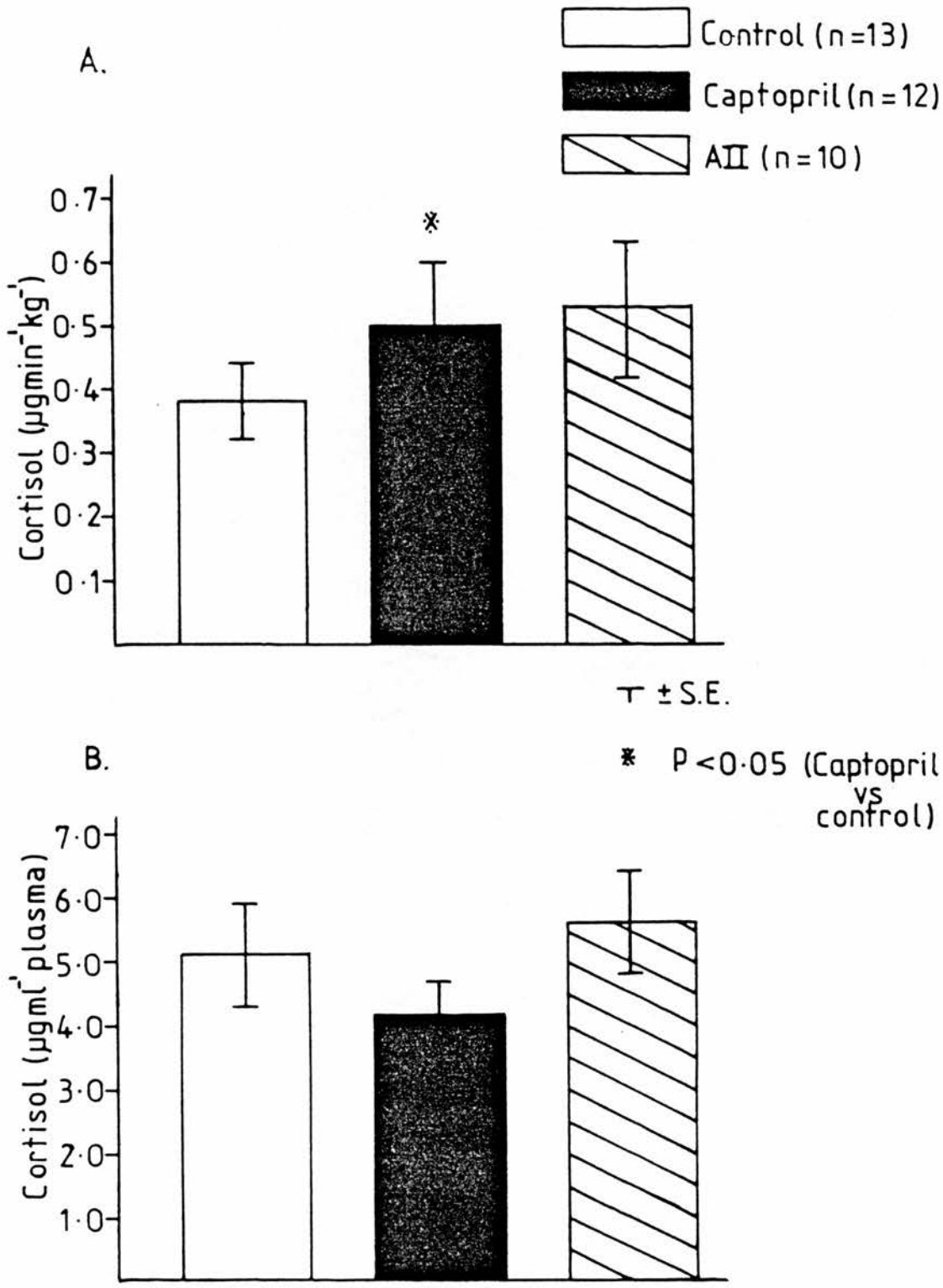
Table 2.8

Adrenal cortisol concentration ($\mu g ml^{-1}$ plasma) and output ($\mu g min^{-1} kg^{-1}$) in dogs 13-18, before and after captopril administration, and after a subsequent infusion of angiotensin II (AII).

Dog number and drug treatment	Cortisol concentration	Plasma volume per minute	Cortisol output
Control			
13	4.63,4.56	2.8,2.8	0.52,0.51
14	8.63,10.4,9.99	1.1,1.0,1.7	0.53,0.58,0.94
15	5.39,4.74	0.8,1.2	0.17,0.22
16	2.69,1.87	2.4,3.6	0.30,0.30
17	2.61,2.1	2.5,2.5	0.31,0.25
18	3.99,4.71	1.3,0.8	0.17,0.13
Mean ± SE (n=13)	5.10 ± 0.79	1.88 ± 0.26	0.38 ± 0.06
Captopril			
13	3.38,2.84	4.6,4.6	0.62,0.52
14	5.89,8.27	3.9,2.4	1.28,1.09
15	2.92,2,28	1.9,1.6	0.21,0.14
16	2.75,2.42	3.3,2.8	0.41,0.31
17	3.90,5.34	2.5,1.7	0.47,0.43
18	4.73,4.94	1.5,1.8	0.28,0.25
Mean ± SE (n=12)	4.13 ± 0.51	2.72 ± 0.33	0.50 ± 0.10
AII ($5 ng min^{-1}$)			
13	6.39,6.73	2.4,2.0	0.61,0.54
14	10.7,6.12	2.0,3.7	1.19,1.25
15			
16	4.32,2.94	1.5,2.3	0.30,0.31
17	5.05,8.09	1.3,1.2	0.31,0.46
18	2.40,3.11	1.8,1.8	0.14,0.19
Mean ± SE (n=10)	5.59 ± 0.82	2.0 ± 0.23	0.53 ± 0.12

Figure 2·3

The effect of captopril (25mg) and angiotensin II [AII] (5ngmin^{-1}), in the presence of captopril, on the output of cortisol (A.) and the concentration of cortisol (B.) from the left adrenal gland of the anaesthetised dog.



Comment

These results show that captopril had opposing effects on ACS output and concentration, increasing ACS output and decreasing ACS concentration. Whether it is the output of ACS from the adrenal gland or the concentration of ACS arriving at the adrenal medulla, that is important in the postulated stimulation of adrenal catecholamine release will be discussed in the "discussion" section.

7. The effect of ACTH administration on cortisol secretion

The output and plasma concentration of cortisol in adrenal venous blood 10-40 minutes after ACTH administration was analysed in dogs 48, 49 and 51. The results are shown in table 2.9.

No statistical analysis of these results was possible due to the limited values available, but the results show that there was an increase in cortisol output and concentration 10 minutes after ACTH administration, and indicate that ACTH-induced cortisol secretion was greatest 20-40 minutes after ACTH administration. In dogs 7, 8, 9, 11 and 12 the first baroreceptor tests were carried out 20-30 minutes after ACTH administration. These results indicate that at the time the baroreceptor tests were performed, steroid secretion had been stimulated by ACTH.

8. The effect of ACTH on adrenal blood flow

The effect of ACTH on adrenal blood flow was analysed in dogs 8,

Table 2.9

The effect of ACTH administration on adrenal cortisol output

		Time after ACTH administration (min)				
	Dog number	0	10	20	30	40
Cortisol output ($\mu g min^{-1} kg^{-1}$)	48	0.57	0.80	0.84	0.66	0.77
	49	0.50	0.47	0.57	0.59	0.68
	51	0.23	0.38	0.67	0.60	
	Mean ± SE	0.43 ± 0.10	0.55 ± 0.13	0.69 ± 0.08	0.62 ± 0.02	0.73
	Dog number					
Cortisol concentration ($\mu g ml^{-1}$ plasma)	48	13.1	13.1	14.8	13.8	16.2
	49	4.21	3.96	5.05	5.37	6.20
	51	4.02	4.62	6.69	4.91	
	Mean ± SE	7.11 ± 3.0	7.23 ± 2.94	8.85 ± 3.0	8.03 ± 2.89	11.2

9, 11, 48, 49, 50 and 51. The results are shown in table 2.10.

The results show that ACTH did not induce a significant increase in adrenal blood flow, although the results indicate that there may be a tendancy for ACTH to increase adrenal blood flow.

9. The effect of adrenal blood flow on cortisol and corticosterone output

The effect of adrenal blood flow on cortisol and corticosterone output was analysed. A plasma volume vs corticosteroid output graph was constructed. The linear regression lines with 95% confidence limits are shown in figure 2.4. The control data used to compile this figure can be found in tables 2.7 and 2.8.

Figure 2.4 shows that corticosteroid output is not significantly dependant on adrenal blood flow.

10. The relationship between cortisol output and concentration on adrenal catecholamine release

In dogs 29, 49 and 51 ACTH, was administered and catecholamine and simultaneous cortisol output was monitored. It was therefore possible to analyse the relationship between cortisol output and concentration and catecholamine release in these dogs. The results are illustrated in figures 2.5 and 2.6 which show linear regression lines

Table 2.10

The effect of ACTH on adrenal blood flow

	Adrenal blood flow ($ml min^{-1}$)				
Time (min) after administration of ACTH	0	10	20	30	40
Dog number					
8	4.8	10.7	8.0	9.0	
9	3.0	2.6	4.4	3.0	
11	4.0	4.6	4.7	5.0	
48	1.5	2.2	1.8	1.7	1.6
49	4.4	4.3	3.9	3.8	3.7
50	4.2	5.4	5.5	6.2	5.0
51	2.3	2.8	2.3	3.1	3.0
Mean ± SE	3.7 ± 0.46 (n=7)	4.66 ± 1.10 (n=7)	4.37 ± 0.78 (n=7)	4.54 ± 0.92 (n=7)	3.33 ± 0.71 (n=4)

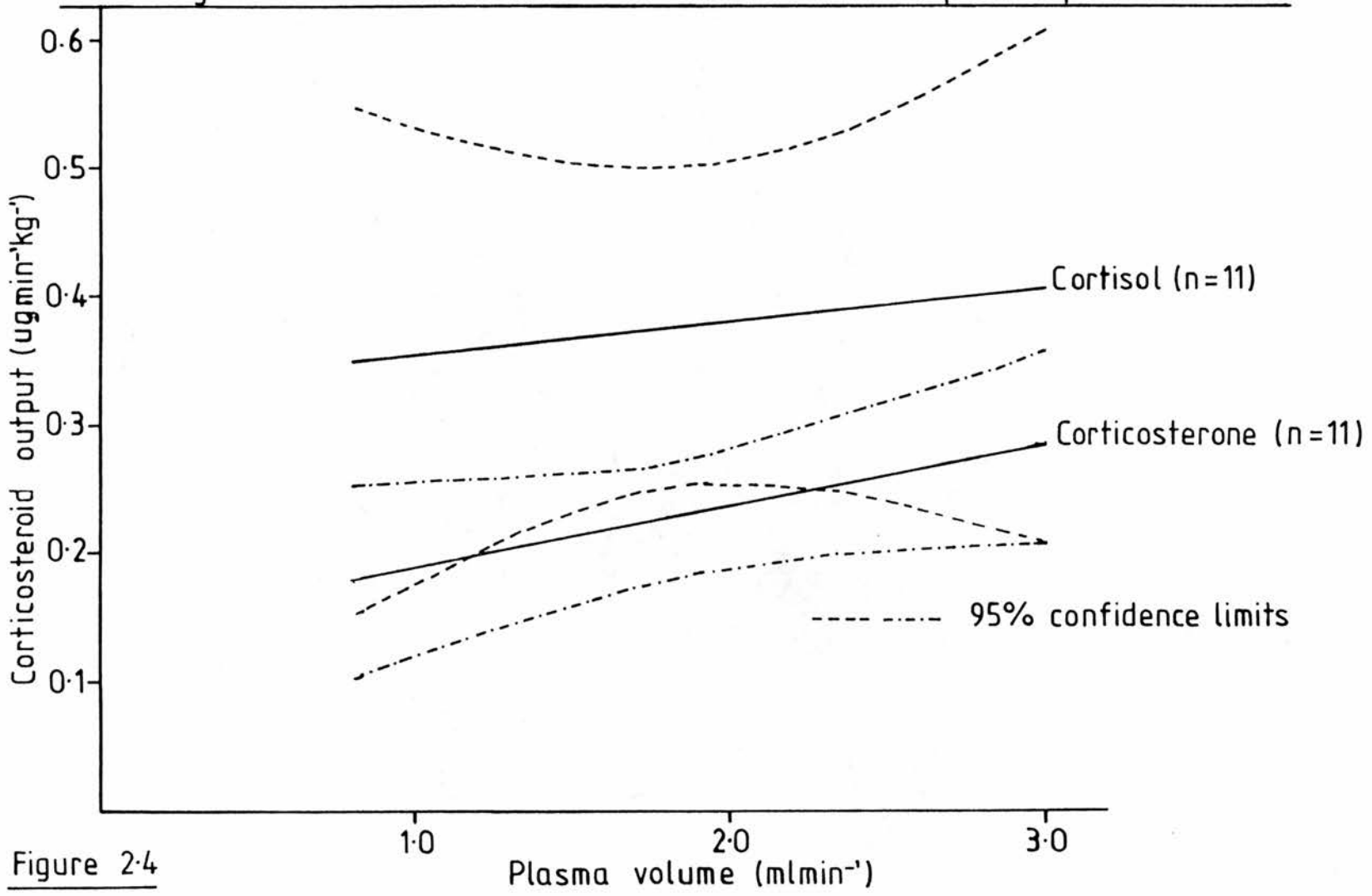

Linear regression lines for corticosterone and cortisol output vs plasma volume
0·6
0·5
0·4
0·3
0·2
0·1
Corticosteroid output (ugmin⁻¹kg⁻¹)
Cortisol (n=11)
Corticosterone (n=11)
95% confidence limits
1·0
2·0
3·0
Plasma volume (mlmin⁻¹)

Figure 2·4

Figure 2·5

Linear regression line for cortisol output vs. catecholamine output, from the left adrenal gland.

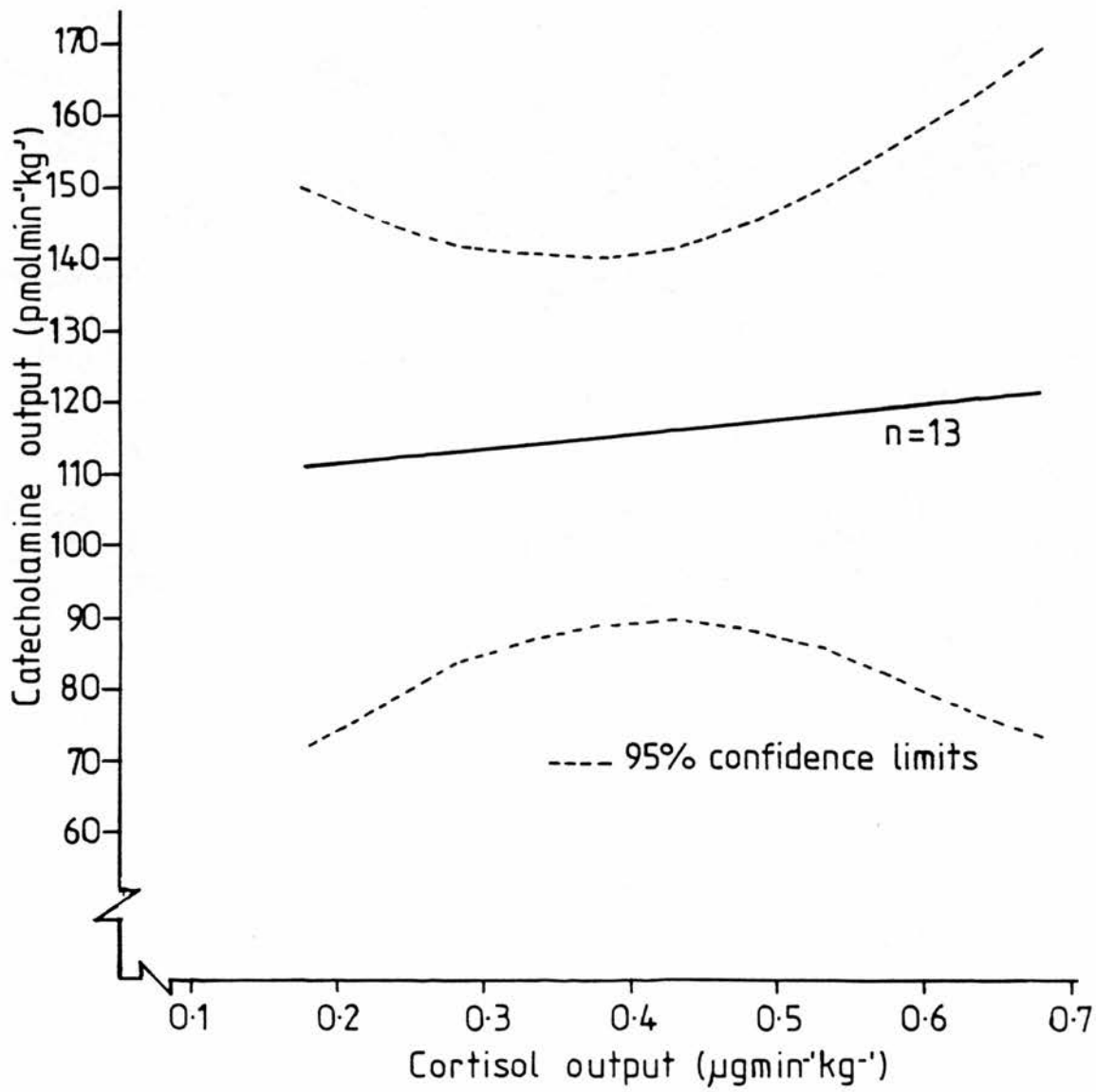
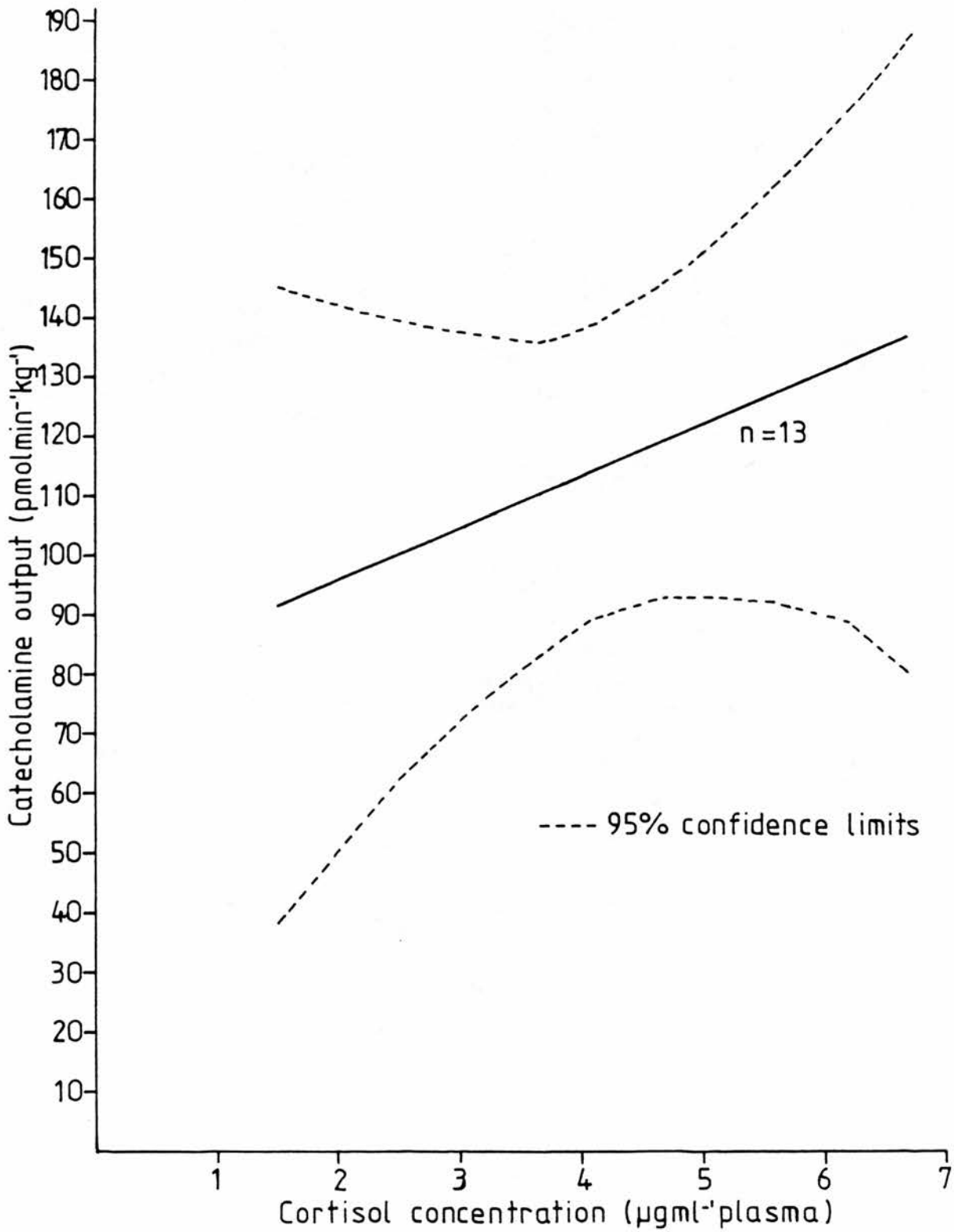


Figure 2·6

Linear regression line for cortisol concentration in adrenal venous blood plasma vs. adrenal catecholamine output



with 95% confidence limits for cortisol output and concentration (prior to captopril administration), respectively, vs catecholamine output. These figures were constructed from the data contained in table 2.11. Figures 2.5 and 2.6 show that the large variation in catecholamine output between animals resulted in large 95% confidence limits. Figure 2.5 indicates that cortisol output is not related to catecholamine output. Figure 2.6 indicates that, although not significant, there is a trend towards increasing plasma cortisol concentrations increasing adrenal catecholamine output.

11. The effect of ACTH on the ratio of adrenaline:noradrenaline in adrenal venous blood

As has been discussed in the "Introduction and literature review", the effect of adrenocorticosteroids on adrenal catecholamine secretion may be secondary to an effect on the enzyme PNMT, which converts noradrenaline to adrenaline. If the effect was on PNMT alone, ACTH should only affect adrenaline release, and there would be evidence of an increase in the ratio of adrenaline:noradrenaline following ACTH administration.

The effect of ACTH on the ratio adrenaline:noradrenaline in dogs 29, 49 and 51 was analysed. The results are shown in table 2.12.

The results show that ACTH did not increase the ratio of adrenaline:noradrenaline and hence its effect on catecholamine release is not due to induction of PNMT by adrenocorticosteroids.

Table 2.11

Catecholamine output, cortisol output and cortisol concentration before and after ACTH administration, in dogs 29, 49 and 51

	Drug Treatment	Sample number	Dog 29	Dog49	Dog51
Catecholamine output ($pmol\,min^{-1}\,kg^{-1}$)	Control	1.	52	86	88
		2.	58		93
	+ ACTH	1.	118	112	130
		2.	178	111	
		3.	223	111	
		4.		141	
Cortisol output ($\mu g\,min^{-1}\,kg^{-1}$)	Control	1.	0.18		0.30
		2.	0.19	0.50	0.23
	+ ACTH	1.	0.23	0.47	0.67
		2.	0.23	0.57	
		3.	0.23	0.59	
		4.		0.68	
Cortisol concentration ($\mu g\,ml^{-1}$ plasma)	Control	1.	1.49		4.02
		2.	2.29	4.21	4.87
	+ ACTH	1.	3.36	3.96	6.69
		2.	3.70	5.05	
		3.	3.36	5.37	
		4.		6.20	

Table 2.12

The effect of ACTH on the ratio adrenaline:noradrenaline in adrenal venous blood

	Ratio adrenaline:noradrenaline			
	Dog 29	Dog 49	Dog 51	Mean ± SE (n=10)
Control	5.13 2.70 4.80	4.16 4.44 4.75 4.45	6.30 4.80 5.10	4.66 ± 0.29
+ ACTH	2.70 4.70 3.50	4.76 4.84 4.43 4.70	6.30 5.20 5.30	4.64 ± 0.31

Part 2 - Summary of results

1. Cycloheximide ($50 mg kg^{-1}$), administered after captopril and during AII infusion inhibited resting adrenal catecholamine release and totally abolished the reflex release induced by baroreceptor stimulation. The same dose of cycloheximide, administered before captopril, did not inhibit the resting release of catecholamines and reduced, and did not abolish the reflex release. This indicates that inhibition of adrenocorticosteroid secretion, in response to ACTH, by cycloheximide and inhibition of AII synthesis by captopril both independantly inhibit adrenal catecholamine release in response to baroreceptor stimulation. When both adrenocorticosteroid secretion and AII synthesis are inhibited simultaneously, despite infusion of a low level of AII, the reflex release of catecholamines is abolished.

2. Cycloheximide had no effect on resting systemic blood pressure, or the reflex pressor response to baroreceptor stimulation.

3. ACTH ($100 \mu g$) partially reversed the inhibitory effect of captopril on reflex adrenal catecholamine release in response to baroreceptor stimulation. ACTH-induced steroid secretion had increased by the time baroreceptor tests were performed.

4. ACTH had no effect on systemic blood pressure but may have prevented the further fall in systemic blood pressure usually observed some time after captopril administration.

5. Captopril did not affect adrenal corticosterone output, but

significantly reduced corticosterone concentration in adrenal venous blood. Captopril increased adrenal cortisol output and reduced cortisol concentration in adrenal venous blood.

6. ACTH did not significantly increase adrenal blood flow although there was a tendency towards an increase following ACTH administration.

7. Cortisol and corticosterone output, prior to captopril administration, was independent of adrenal plasma volume.

8. Adrenal catecholamine output was not related to adrenal cortisol output, although there was a tendency for an increase in cortisol concentration in adrenal venous blood to induce an increase in adrenal catecholamine release.

9. ACTH did not increase the ratio of adrenaline:noradrenaline in adrenal venous blood and so the increase in adrenal catecholamine release following ACTH administration was not due to induction of PNMT by adrenocorticosteroids.

Part 2 - Discussion of results

Cycloheximide inhibits the release of adrenocorticosteroids (ACS) in response to ACTH (Garren etal, 1965) probably by inhibiting the synthesis of new protein. Roth and Hughes (1972) demonstrated that a pharmacological dose of AII stimulates the biosynthesis of the enzymes involved in noradrenaline synthesis and that cycloheximide abolished AII-induced increases in noradrenaline synthesis, whilst having no effect on basal noradrenaline synthesis. Other studies have, however, demonstrated that the effect of AII on noradrenaline biosynthesis is not seen at physiological levels of AII (see "Part 3 - Introduction and literature review").

In dog 10, it was seen that cycloheximide, administered before captopril did not inhibit the resting output of catecholamines but did inhibit the reflex release in response to baroreceptor stimulation. If the effect of cycloheximide on new protein synthesis is considered, the results could indicate that cycloheximide was inhibiting catecholamine release through inhibition of the enzymes involved in de novo biosynthesis of catecholamines. This is unlikely, however, as considerable ammounts of catecholamines are stored in the adrenal medulla and so release does not depend on de novo biosynthesis. In addition, if this was the case, cycloheximide alone would be expected to completely abolish reflex catecholamine release, and this was not observed. Critchley etal (1982) also demonstrated that cycloheximide reduced, though not significantly, reflex adrenal catecholamine release.

The results suggest, therefore, that inhibition of ACTH-induced ACS secretion may inhibit reflex catecholamine release, and that ACS may exert a facilitatory effect on reflex catecholamine release.

In dogs 2-5, cycloheximide was administered after captopril, and during the AII infusion. It severely inhibited resting release of catecholamines and totally abolished the residual reflex release of catecholamines remaining after captopril administration.

This suggests that when both ACTH-stimulated ACS secretion and AII synthesis are inhibited, the independant inhibitory effects of captopril and cycloheximide are potentiated, resulting in virtually total inhibition of reflex catecholamine release. This inhibition is so severe, that the level of AII which normally reverses the effect of captopril was unable to overcome it.

Our hypothesis was that AII may exert a facilitatory effect on ACS secretion which in turn may exert a facilitatory effect on catecholamine release. As I have shown and will be discussed later, captopril does reduce corticosterone and cortisol concentration in adrenal venous blood, and catecholamine release tends to increase with increasing cortisol concentration. In addition, as was discussed in the "Introduction and literature review", ACTH and corticosteroids have been shown to stimulate adrenal catecholamine release. This evidence, together with the results discussed for cycloheximide effects, supports our hypothesis.

The evidence suggests that captopril may inhibit a direct

facilitatory effect of AII on adrenal catecholamine release (see Feldberg and Lewis, 1964; Peach, 1971; Starke, 1972; Haefely, 1972; Reit, 1972 and "Part 1" of this thesis) and also an indirect facilitatory effect of AII via ACS secretion, which may exert a facilitatory effect on adrenal catecholamine release.

As will be discussed later, there is evidence that the facilitatory effect of AII on cortisol secretion may be only effective in the presence of ACTH (Slater etal, 1963; Bravo etal, 1975; Parker etal, 1983; Morera etal, 1984). This may explain why the combined effects of both captopril and cycloheximide abolish the reflex adrenal release of catecholamines, as the combined effects of ACTH and AII had both been inhibited.

The results indicate that the proposed facilitatory effect of ACS on catecholamine release is predominantly on the reflex release induced by the baroreceptor tests, as cycloheximide had little effect on resting catecholamine release. This is supported by my results which show that ACTH reverses the inhibitory effect of captopril on reflex release of catecholamines, whilst having little effect on the resting release (the results also show that there was ACTH-stimulated ACS secretion by the time baroreceptor tests were performed). It is also supported by the studies of others which have demonstrated ACTH release from the pituitary and subsequent ACS secretion following chemoreceptor stimulation (Anichkov etal, 1960; Marotta, 1972), insulin- hypoglycaemia (Wurtman etal, 1968) and haemorrhage (Ganten etal, 1979; Lilly etal 1982). This evidence and that described in the "Introduction and literature review", combined with my own, suggest

that both the renin-AII system and the pituitary adrenocortical axis are important in the adreno-medullary response to hypotension. They may also facilitate the effects of each other, cooperating to restore eg. blood volume. My results also show that ACTH alone may have prevented the further fall in blood pressure usually observed after captopril administration. They also show that ACTH did not increase the ratio of adrenaline:noradrenaline in adrenal venous blood and so the effects of ACTH on adrenal catecholamine release cannot be explained by ACS induction of PNMT.

If the hypothesis that AII is exerting an indirect effect on adrenal catecholamine release through facilitation of ACS is correct, captopril would be expected to reduce ACS secretion from the adrenal cortex.

The results show that captopril did significantly reduce corticosterone plasma concentration, and reduced cortisol plasma concentration to a lesser extent. There was an indication, although not significant, that AII partially reversed these effects. Captopril did, however, have no effect on corticosterone output and significantly increased cortisol output.

It must be considered, therefore, which is the most important in stimulating catecholamine release, the concentration of ACS in the adrenal blood, or the total ACS output.

As I described in "An introduction to the adrenal gland (blood supply)" blood passing through the sinuses connecting the cortex with

the medulla, comes into intimate contact with chromaffin cells and, as described by Coupland (1975), these cells are affected by high concentrations of ACS which accumulate in the immediate vicinity of the cells, and it is this concentration that effects catecholamine synthesis. I therefore consider that it is the plasma concentration of ACS which will affect catecholamine synthesis, and that the reduction of this by captopril contributes to its inhibitory effect on catecholamine output. In addition, it has been shown that captopril significantly increased adrenal blood flow, and as ACS output was calculated by multiplying concentration by blood flow, these increases in adrenal blood flow were responsible for the apparent increases in ACS output.

The results therefore indicate that captopril may reduce the postulated facilitatory effect of cortisol and corticosterone on catecholamine secretion, by reducing the plasma concentration of cortisol and corticosterone bathing the chromaffin cells.

Figures 2.5 and 2.6 show that after ACTH administration, although not significant, there is a tendency for catecholamine output to increase with an increase in plasma cortisol concentration but not with cortisol output, which also indicates that it is the concentration which is important.

These results are supported by the work of Critchley etal (1982) who demonstrated that cycloheximide abolished the prolonged adrenal release of catecholamines stimulated in response to carotid body hypoxia in the anaesthetised dog. As described in the "Introduction

and literature review", Critchley etal (1982) also showed that the results of Wurtman etal (1968) could be recalculated to show that ACTH stimulated release of adrenaline and noradrenaline and so the effect was not totally related to induction of PNMT as Wurtman etal suggested. Critchley etal (1975) had also demonstrated that cortisol could stimulate release of catecholamines from the isolated perfused canine gland in a dose-dependant manner and in concentrations normally detected in adrenal venous blood.

Edwards, Hardy and Malinowska (1975) and Bloom, Edwards and Hardy (1977) reported that in the conscious calf, cortisol release during hypoxia or hypercapnia was not related to catecholamine release. Henderson (1980) reported that no catecholamine output was detected after cortisol infusion in feline adrenal glands. The effect of corticosteroids on adrenal catecholamine release may therefore be species dependant.

My results and the additional evidence discussed, suggest that, in the anaesthetised dog, stimulation of the pituitary adrenocortical axis in response to cardiovascular stress induces an increase of cortisol and corticosterone from the adrenal cortex which facilitates catecholamine release from the adrenal medulla. They also suggest that this effect is facilitated by the presence of AII which facilitates catecholamine release by a direct action on the adrenal medulla, and indirectly through facilitating adrenocorticosteroid release from the adrenal cortex.

From figures 2.2 and 2.3, it can be seen that captopril

significantly reduced corticosterone plasma concentration, while reducing, though not significantly to $p > 0.05$, cortisol concentration. Neither of these effects was significantly reversed by the low level of AII infused, although there was a tendancy towards a reversal. It may be that increasing levels of AII may have exerted a greater effect.

It is of interest that captopril had a greater effect on corticosterone than cortisol concentration. As captopril inhibits AII synthesis, this suggests that AII may have a more profound effect on corticosterone secretion in the dog. This is supported by additional available evidence which I shall now discuss.

The zona glomerulosa of the adrenal cortex is the site for aldosterone secretion (Martin, 1976). Figure 2.7 shows part of the major biosynthetic pathway for aldosterone. From the figure it can be seen that corticosterone is a major precursor for aldosterone synthesis. Corticosterone is also released from the zona glomerulosa. Cortisol is not synthesised or secreted by the zona glomerulosa.

Cortisol is synthesised and secreted by the zona fasciculata of the adrenal cortex (Martin, 1976). Figure 2.8 shows a major biosynthetic pathway for cortisol. From the figure it can be seen that cortisol is ultimately synthesised from corticosterone. In the rat, corticosterone is the major glucocorticoid secreted by the zona fasciculata, but in humans and other animals (including the dog) corticosterone is converted to cortisol which is the main, or only, glucocorticoid secreted (Martin, 1976).

Figure 2·7
Biosynthetic pathway for aldosterone in the zona glomerulosa.

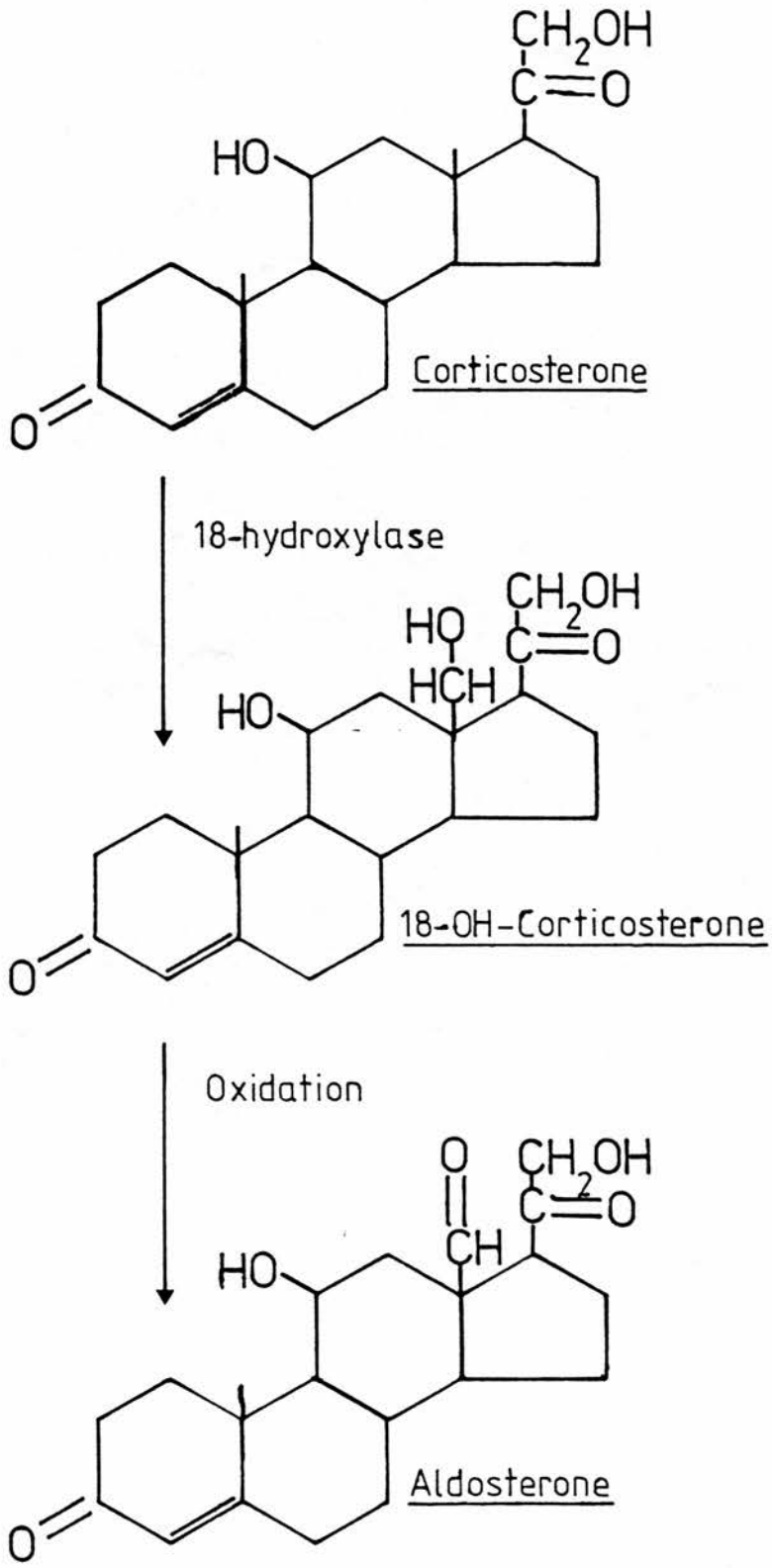
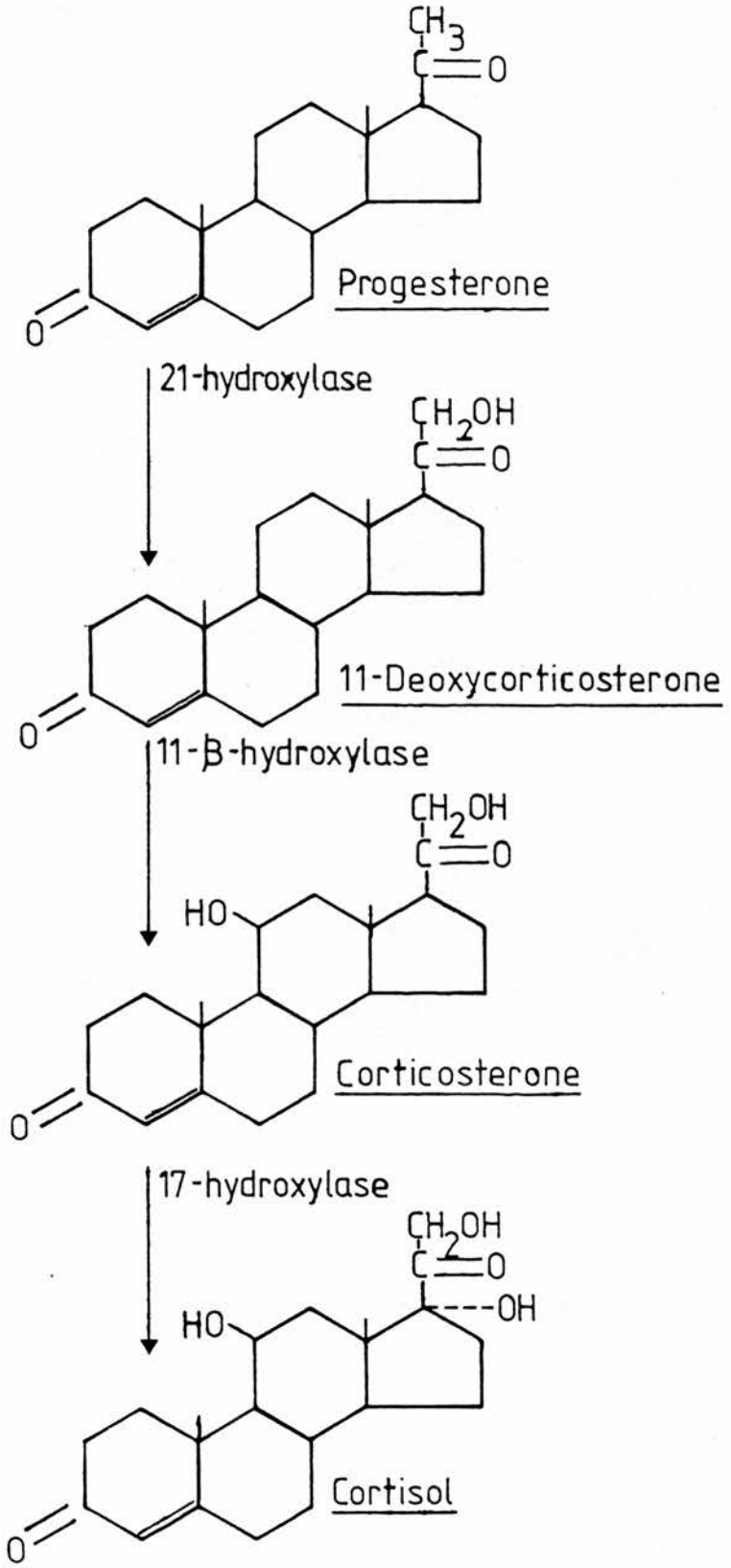


Figure 2·8

Biosynthetic pathway for corticosterone and cortisol in the zona fasciculata.



Aldosterone and corticosterone secretion from the zona glomerulosa is primarily determined by AII, K^+ and ACTH (Huijmans, Falke and Degenhart, 1984) and the stimulation of secretion is Ca^{++}-dependant (Braley, Menachey, Brown and Williams, 1984). Braley and Williams (1977) and Shima, Kawashima and Hirai (1978) found no evidence of corticosterone stimulation by AII in rat fasciculata cells, but AII did stimulate corticosterone from the glomerulosa cells.

Cortisol secretion from the zona fasciculata is mainly ACTH dependant (Parker etal, 1983; Huijmans etal, 1984). AII may also play a role in cortisol secretion from the zona fasciculata in some species. Slater etal (1963) and Bravo etal (1975) demonstrated that AII may play a role in cortisol secretion in the dog. Carpenter etal (1961) and Kaplan and Bartter (1962) also demonstrated that AII stimulates cortisol secretion in bovine and dog adrenals.

The evidence therefore suggests that, in the dog, AII is a major stimulus for corticosterone secretion from the zona glomerulosa and plays a minor role in cortisol secretion from the zona fasciculata. This may explain why I found that captopril reduced corticosterone adrenal plasma concentration to a more significant extent than cortisol concentration.

As mentioned, the effect of AII on cortisol secretion may be species dependant. McKenna, Island, Nicholson and Liddle (1978) demonstrated that pharmacological doses of AII stimulated cortisol production in slices of adrenal tissue from patients with Cushings

disease. Williams and Braley (1977) were unable to demonstrate cortisol production in response to physiological doses of AII, in collagenase-dispersed human fasciculata cells. So it is not clear whether AII does play a role in cortisol secretion from human fasciculata cells.

Douglas etal (1978) found that AII receptors are significantly more concentrated in rat glomerulosa than fasciculata. This would indicate that AII receptors do exist in rat fasciculata, although Braley and Williams (1977) and Shima etal (1978) found no evidence of corticosterone secretion by AII in rat fasciculata cells. Valloton, Capponi and Grillet (1981) found evidence of AII receptors in bovine fasciculata cells, and Douglas etal (1984) found evidence of an AII receptor, on human and primate collagenase adrenal fasciculata, that shares many properties with the glomerulosa receptor site.

So it would appear that the effect of AII on cortisol secretion may be species dependant. There is evidence that AII may only be effective in stimulating cortisol secretion when it is in the presence of ACTH. Parker etal (1983), using dog adrenal cell suspensions, demonstrated that cortisol secretion was not stimulated by AII alone, but it was stimulated when AII (4×10^{-10}M) was combined with ACTH (10^{-13}M) and these are both physiological concentrations. Slater etal (1963) and Bravo etal (1975) also demonstrated that, in the dog, AII potentiates the response of ACTH on cortisol secretion. Morera etal (1984) demonstrated that AII potentiates ACTH-induced cyclic AMP production in bovine cortico- adrenal cells, an effect which was inhibited by saralasin. Fraser etal (1978) demonstrated that cortisol

production, in dexamethasone supressed normal volunteers, was not stimulated by AII alone, but was when AII was infused over a baseline of ACTH stimulation. These observations may explain why the studies using in vitro fasciculata cell preparations were unable to demonstrate cortisol secretion by AII alone.

My observation that cycloheximide potentiated the effects of captopril on adrenal catecholamine release are compatible with a potentiation of ACTH induced cortisol secretion by AII.

The results show that cortisol output was approximately two times that of corticosterone output. This was also observed by Tait, Tait and Bell (1980) who demonstrated that steroid production rates in fasciculata cells in the presence of ACTH or other stimuli exceed the production rates observed in glomerulosa cells.

The results in table 2.10 show that ACTH did not promote a significant increase in adrenal blood flow, although there was a tendancy towards an increase. Other workers have found that ACTH, at doses which stimulate cortisol secretion have little effect on adrenal blood flow (Coupland, 1975), although others have detected significant increases in adrenal blood flow following ACTH administration. There are also conflicting opinions as to the effect of ACTH-induced increases in adrenal blood flow on ACS secretion. Figure 2.4 shows that, prior to captopril administration, cortisol and corticosterone output was not related to adrenal blood flow. This indicates that ACS release is not dependant on diffusion into adrenal blood. Marotta (1972) studied the release of 11-hydroxycorticosteroids (11-OHCS)

(cortisol and corticosterone (see figure 2.8)) in the intact anaesthetised dog made hypoxic or infused with ACTH. He detected an increase in 11-OHCS secretion within two minutes of applying the stimuli, and this was accompanied by an increase in adrenal blood flow. He suggested that the sudden increase in adrenal blood flow may rapidly release preformed steroids stored in the adrenal cortex. He did, however, discuss that the increase in adrenal blood flow could not alone account for the elevation in 11-OHCS, since in the hypoxic dogs, the increase in flow was small compared to the increase in secretory rates. This is compatible with my own findings. Marotta (1972) also demonstrated that in dogs infused with acetyl-β-metacholine (AM) (which stimulates the central pole of the hypothalamus), adrenal blood flow had decreased before peak 11-OHCS secretory rates had been reached. L'Age, Gonzalez-Luque and Yates (1970) also demonstrated that in dexamethasone treated dogs, AM caused an increase in adrenal blood flow without appreciably affecting cortisol secretion, and so the release of steroids following AM administration is independant of its effect on adrenal blood flow. The available evidence suggests that an increase in adrenal blood flow may not be important for ACS secretion from the adrenal cortex.

The results shown in table 2.3 show that cycloheximide administration, in the presence of captopril and AII, had no effect on the reflex pressor response to baroreceptor stimulation, although it abolished the reflex release of catecholamines. This provides indirect evidence that the combined facilitatory effects of the renin-AII system and the pituitary adrenocortical axis is at the level of the adrenal gland, and not on central activation of sympathetic drive.

If this was the case, the reflex pressor response to baroreceptor stimulation would also have been inhibited by cycloheximide.

In conclusion, these results indicate that, in the anaesthetised dog, in addition to a direct effect on adrenomedullary catecholamine release, AII may exert an indirect effect through stimulation of adrenocorticosteroids. These may then facilitate adrenal catecholamine release. In situations of cardiovascular stress, both the renin-AII system and the pituitary adrenocortical axis may cooperate to increase adrenal catecholamine release until homeostasis is restored.

Part 3

It is possible that captopril may inhibit the release of catecholamines from the adrenal medulla via an action on splanchnic nerve activity.

Soon after synthetic AII became available, it was found to facilitate sympathetic neuroeffector transmission (Zimmerman, 1962; McCubbin and Page, 1963; Benelli, Bella and Gandini, 1964). For a review of the effects of AII on the sympathetic nervous system, I refer the reader to (Starke, 1977a). I shall discuss here the evidence supporting a facilitatory role of AII in sympathetic neuro-effector transmission and the possible implications for a similar role for AII in facilitating splanchnic nerve stimulation of catecholamine release from the adrenal medulla.

AII could facilitate sympathetic neuro-effector transmission via a number of mechanisms-:

1. By a post-synaptic effect on the sensitivity of effector tissues to catecholamines.
2. By a presynaptic effect on basal catecholamine release.
3. By a presynaptic effect on stimulation-evoked release of catecholamines.
4. By a presynaptic effect on neuronal uptake of catecholamines.
5. By an effect on noradrenaline biosynthesis in nerve terminals.

1. Facilitation of the sympathetic nervous system by AII has been shown to be partly due to a direct action on the effector organ eg.

smooth muscle cells (Panisset and Bourdois, 1968; Day and Moore, 1976). At low doses AII causes a partial depolarisation of cell membranes, bringing the cells closer to the firing level of action potentials (Sjostrand and Swedin, 1974). AII can facilitate both pre- and post-junctional effects of adrenergic transmission in the rat mesenteric bed (Malik and Nasjletti, 1976; Zimmerman, 1978; Campbell and Jackson, 1979). The vasoconstriction of the rat mesenteric bed induced by exogenous noradrenaline can be blocked by angiotensin converting enzyme inhibitors (ACEIs) (Saruta, Suzuki, Okimo and Kondo, 1982; Clough etal, 1982).

Clough etal (1982) demonstrated that, in pithed rats, the pressor responses induced by exogenous noradrenaline can also be reduced by ACEIs and saralasin. In this preparation ACEIs and saralasin also reduced the pressor responses induced by stimulation of the sympathetic nervous system. This indicates that inhibitors of the renin-AII system may inhibit neurogenic vasoconstriction by interfering with both pre- and post-junctional effects of AII.

2. Hughes and Roth (1971), using the rabbit coeliac artery preincubated with ^{3}H noradrenaline, showed that as little as $10^{-10}M$ AII accelerated the basal outflow of tritium. The effects on the noradrenaline overflow could be retained after neuronal and extra-neuronal uptake of noradrenaline was blocked. This indicates a facilitatory effect of AII on basal release of noradrenaline. In many other tissues, however, a high concentration of AII is required to obtain even small increases in basal overflow (see Starke, 1977a).

Much of the available evidence indicates that the most important facilitatory effect of AII is on stimulation-evoked release of noradrenaline.

3. Zimmerman and Whitmore (1967) first showed that AII augments the overflow of noradrenaline evoked by sympathetic nerve stimulation. AII enhances the stimulation-evoked overflow of noradrenaline from the hind paw, kidney of the dog, rabbit heart, coeliac and pulmonary arteries, portal vein and vas deferens. Studies using tissues pre-labelled with tritiated noradrenaline have shown that AII enhances the stimulation-evoked overflow of radioactivity (see Starke, 1977a). This prejunctional effect of AII is probably its most important facilitatory effect on sympathetic nerve activity, as it enhances the release of noradrenaline by nerve impulses at doses which do not affect the basal overflow of noradrenaline and do not affect the release of noradrenaline induced by tyramine or amphetamine, and therefore the uptake of noradrenaline by nerve terminals (Starke, 1971; Chevillard and Alexandre, 1972). The effect on stimulated release of noradrenaline is not secondary to an enhancement of noradrenaline synthesis since release of exogenously administered noradrenaline is also increased (see Starke, 1977a). Not only blood-borne, but also locally formed AII may facilitate noradrenaline transmission (Malik and Nasjletti, 1976). There is evidence that the presynaptic AII receptor may differ from the myocardial and smooth muscle AII receptor (Zimmerman, 1973; Blumberg, Ackerly and Peach, 1975).

The reason that some workers have been unable to demonstrate a

facilitatory effect of AII on prejunctional noradrenaline release may be because the facilitatory effect of AII is pronounced at low frequencies of stimulation, but declines at high frequencies (Hughes and Roth, 1971; Henderson and Hughes, 1974). This is compatible with the view that AII selectively enhances Ca^{++} dependant secretion. Bell (1972), using electrophysiological studies on guinea-pig vas deferens and uterine artery, found that the extra-junctional potential (e.j.p.) evoked by a single nerve impulse was not enhanced by AII. AII did facilitate the effects of successive e.j.p.s during low frequency stimulation. If it is assumed that facilitation in a train of impulses reflects increased intra-neuronal levels of Ca^{++}, until at high frequencies the "release receptors" are saturated, then these results indicate that AII promotes the ammount of Ca^{++} available for release processes (Starke, 1972).

AII has also been shown to facilitate the average release of noradrenaline per nerve impulse (Zimmerman, Gomer and Chialiao, 1972). It has also been shown, in the rabbit atria, that AII enhances the overflow of both noradrenaline and dopamine-β-hydroxylase, which is a marker enzyme for storage vesicles (Ackerly, Blumberg and Peach, 1976). This suggests that AII enhances Ca^{++}-dependant exocytotic release of noradrenaline.

4. Studies concerning the effects of AII on neuronal uptake of noradrenaline have yielded contradictory results, even in the same experimental organ (see Starke, 1977a), and it seems unlikely that, at physiological concentrations, AII exerts an inhibition on neuronal uptake mechanisms. Only at very high concentrations eg. greater than

10^{-5}M, AII slightly decreases neuronal uptake (Schumman, Starke, Werner and Hellerforth, 1970; Henderson and Hughes, 1974) and an appreciable effect of lower concentrations is doubtful (Thoenen, Hurlimann and Haefely, 1965; Gommer and Zimmerman, 1973; Day and Moore, 1976). Up to 5 x 10^{-6}M AII fails to reduce the release of noradrenaline by tyramine (Starke, 1971; Chevillard and Alexandre, 1972) and AII has actually been shown to enhance the post-synaptic effects of tyramine (Benelli etal, 1964; Kaneko, Takeda, Nakajima and Ueda, 1966; Chevillard and Alexandre, 1972). The available evidence does not support an effect on neuronal uptake by AII.

5. Roth and Hughes (1972) demonstrated that AII stimulates the biosynthesis of the enzymes involved in noradrenaline biosynthesis. Puromycin and cycloheximide block the synthesis of new protein and abolished AII increases in noradrenaline synthesis, whilst having no affect on basal noradrenaline synthesis.

In the rabbit coeliac artery, vas deferens and many other tissues, AII does not effect noradrenaline synthesis, and it is doubtful if AII exerts this effect physiologically.

As has been discussed in Part 1 of this thesis, AII stimulates catecholamine release from the adrenal medulla. It has also been shown to stimulate activity in other sympathetic ganglia cells (Reit, 1972; Starke, 1972). AII depolarises isolated chromaffin cells (Douglas, Kanno and Sampson, 1967) and, like AII-evoked noradrenaline release from sympathetic nerve endings, AII evoked catecholamine release from the adrenal medulla is Ca^{++}- dependant (Poisner and Douglas, 1966).

It has been demonstrated that AII has analogous effects on cholinergic and noradrenergic nerve endings in that it enhances the stimulation-evoked overflow of acetylcholine but not the basal overflow. It can enhance stimulation-evoked acetylcholine overflow from both sympathetic and parasympathetic ganglia, and the stimulation-evoked acetylcholine overflow from the guinea-pig ileum and cerebral cortex (Panisset, 1968).

The aims of this series of experiments were two-fold-:

1. From the evidence presented here, it appeared possible that AII may facilitate splanchnic nerve activity.
2. It was still not possible to positively rule out the possibility, suggested by Feuerstein etal (1977), that following baroreceptor stimulation, AII was acting centrally to increase sympathetic drive and hence reflex adrenal catecholamine release.

The effect of captopril (and saralasin) on the denervated adrenal gland, and its responses to splanchnic nerve stimulation were studied to investigate these two possibilities.

The experiments were designed to answer the following questions-:

1. Does captopril (and saralasin) inhibit the release of catecholamines evoked by splanchnic nerve stimulation, from the denervated adrenal gland ?
2. If so, does exogenously administered AII restore splanchnic nerve stimulation-evoked catecholamine release ?

Part 3 - Results

1. The effect of the renin-angiotensin system on adrenal catecholamine release in response to splanchnic nerve stimulation

1.a. The effect of the frequency of stimulation of the splanchnic nerve on adrenal catecholamine release in the anaesthetised dog

The effect of the frequency of stimulation of the splanchnic nerve on total catecholamine release, before and after captopril administration and a subsequent infusion of AII, in dogs 19-24 was studied. The results are shown in table 3.1.

The effect of the frequency of stimulation on the change in catecholamine release induced by splanchnic nerve stimulation (10 volts,2ms) was also analysed. The results are shown in table 3.2 and illustrated in figure 3.1.

The results show that there was an increase in adrenal catecholamine release with an increase in frequency between 2.5 and 10 pulses per second (pps). A maximum release can be seen to occur at 10pps, 20pps having no additional effect. In subsequent experiments, the splanchnic nerve was stimulated at 10pps with 10 volts (v).

Table 3.1

The effect of captopril on total catecholamine release in dogs 19-24 (D19-24) before and after splanchnic nerve stimulation.

Drug Treatment		Total catecholamine release ($pmolmin^{-1}kg^{-1}$) D19	D20	D21	D22	D23	D24	Mean ± SE
Control								
	C	152	212	11	124	103	31	105.5 ± 30.7
	+S,2.5pps	122	250	24	93	65	42	99.3 ± 33.6
	C	216	353	6	91	120	11	132.8 ± 54.2
	+S,5.0pps	494	392	54	148	159	75	220.3 ± 73.5
	C	225	439		168	116	29	195.4 ± 68.9
	+S,10.0pps	451	523		275	261	244	350.8 ± 57.0**
	C	304	514	17	168	182	21	201.0 ± 76.7
	+S,20.0pps	477	530	20	208	344	253	305.3 ± 76.4*
Captopril								
	C	196	239	28	83		17	112.6 ± 44.8
	+S,2.5pps	83	240	19	52		33	85.4 ± 40.1
	C	216	510	12	98	186	30	175.3 ± 74.7
	+S,5.0pps	144	446	50	100	115	92	157.8 ± 59.0
	C	99	369	16	113	174	23	132.3 ± 53.2
	+S,10.0pps	82	350	13	147	261	107	160.0 ± 50.7
	C	91	440	29	69	158	33	136.7 ± 63.6
	+S,20.0pps	101	407	22	88	214	185	169.5 ± 55.3*
AII ($5ngmin^{-1}$)	C			28	139	378	78	155.8 ± 77.5
	+S,2.5pps			26	118	467	86	272.5 ± 185
	C			24	177		71	90.7
	+S,5.0pps			47	250		91	129.3
	C			31	262		145	146
	+S,10.0pps			11	330		220	222.3
	C			48	285		248	193.7
	+S,20pps			186	308		299	264.3

+S = During splanchnic nerve stimulation (10v) pps = pulses per second
C = Control
Statistical significance (+S vs C and captopril data vs control data)
* = $p > 0.05$
** = $p > 0.01$

Table 3.2

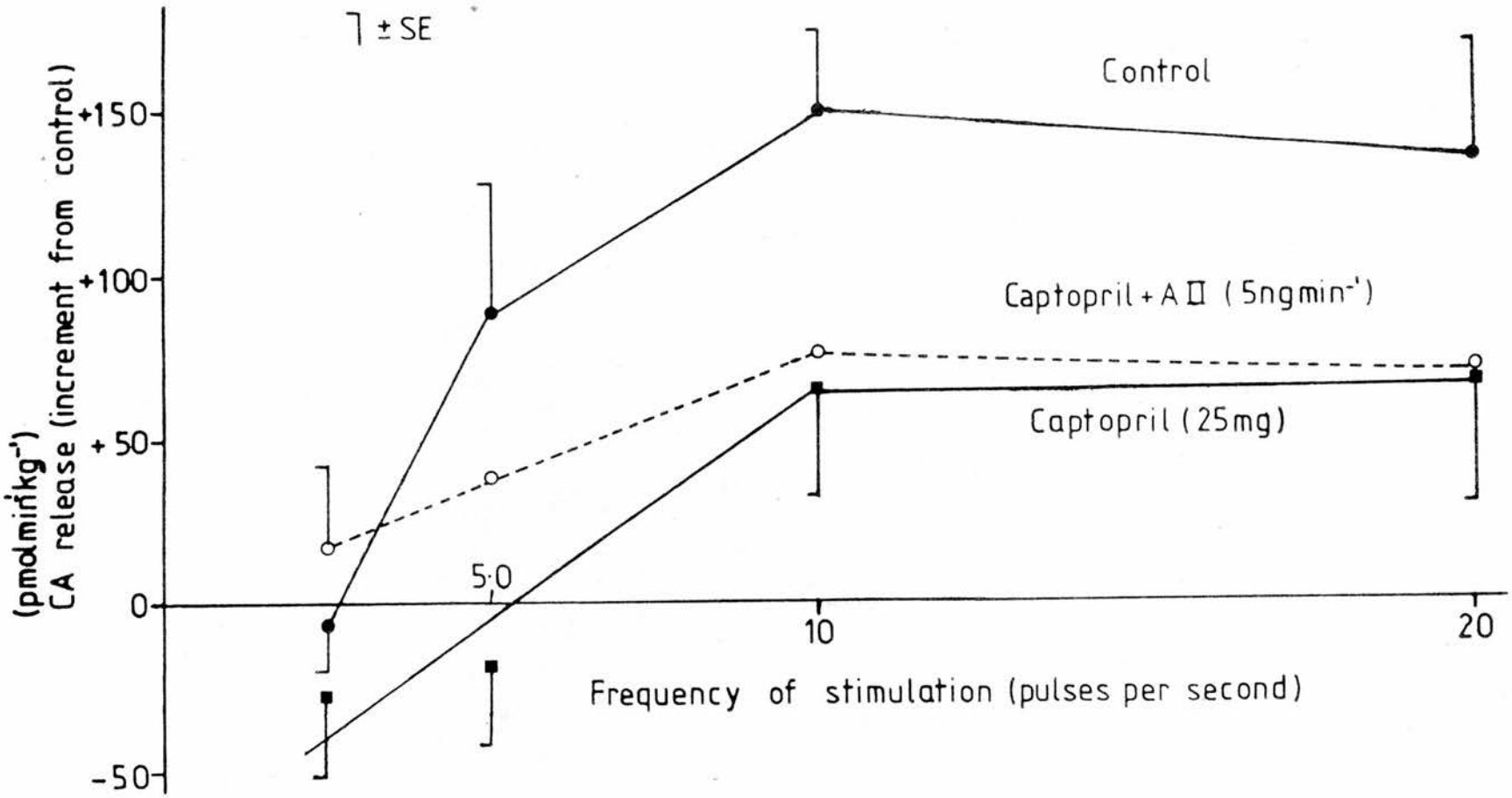
The effect of captopril on the change in catecholamine release induced by splanchnic nerve stimulation in dogs 19-24 (D19-24).

Drug Treatment		Change in catecholamine release ($pmolmin^{-1}kg^{-1}$) D19	D20	D21	D22	D23	D24	Mean ± SE
Control								
	+S,2.5pps	-30	38	13	-30	-38	11	-6.2 ± 12.7
	+S,5.0pps	278	39	48	57	39	64	87.5 ± 38.3
	+S,10.0pps	226	84	121	107	145	215	149.7 ± 23.9
	+S,20.0pps	173	16	190	40	162	232	135.5 ± 35.5
Captopril								
	+S,2.5pps	-113	1	-9	-30		16	-27.0 ± 22.8
	+S,5.0pps	-72	-64	38	2	-71	62	-17.5 ± 24.3
	+S,10.0pps	-17	-19	122	34	87	184	65.2 ± 33.0
	+S,20.0pps	10	-33	198	19	56	152	67.0 ± 36.5*
AII ($5ngmin^{-1}$)	+S,2.5pps			-2	-21	89	8	18.5 ± 24.30
	+S,5.0pps			23	73		20	38.7
	+S,10.0pps			86	68		75	76.3
	+S,20.0pps			138	23		51	70.7

+S = During splanchnic nerve stimulation (10v) pps = pulses per second
C = Control
Statistical significance (captopril data vs control data)
* = $p < 0.05$

Figure 3·1

The effect of frequency of splanchnic nerve stimulation on adrenal catecholamine (CA) release and the effect of captopril followed by angiotensin II (AII) on release evoked by splanchnic nerve stimulation, (10 volts).



1.b. The effect of captopril on adrenal catecholamine release, before and after splanchnic nerve stimulation

The effect of captopril on adrenal catecholamine release, before and after splanchnic nerve stimulation was studied in dogs 25-28. The effect of captopril on total catecholamine release before and after splanchnic nerve stimulation in dogs 25-28 was analysed and pooled with the results obtained from dogs 19-24 for stimulation at 10pps. Table 3.3 shows the results for dogs 25-28, and the mean results for dogs 19-28, and the results are illustrated in figure 3.2. The results show that captopril significantly inhibited both the resting release of catecholamines and the release induced by splanchnic nerve stimulation.

2. The effect of a continuous infusion of AII on adrenal catecholamine release, before and after splanchnic nerve stimulation, and after captopril administration

The effect of a continuous infusion of 5ngmin^{-1} AII on adrenal catecholamine release, before and after splanchnic nerve stimulation was studied in dogs 21,23 and 24. The results for dogs 21,23 and 24 are shown in tables 3.1 and 3.2. In dogs 26-28, continous infusions of 10,30 and 100 ngmin^{-1} AII were administered following captopril administration. These results are shown in table 3.4. The results contained in tables 3.1, 3.2 and 3.4 were combined and illustrated in figure 3.3.

It was not possible to statistically evaluate these results, due to the small sample numbers involved, but the results suggest that

Table 3.3

The effect of captopril on catecholamine release in dogs 25-28 (D25-28) before and after splanchnic nerve stimulation.

Drug Treatment		Total catecholamine release ($pmolmin^{-1}kg^{-1}$) D25	D26	D27	D28	Mean for D19-28 ± SE (n = 11)
Control	C	88,64	58,60	47	23	119.7 ± 36.9
	+S	163,151	72,73	96	106	219.5 ± 45.8***
Captopril	C	64,74	45,41		51,96	79.4 ± 12.7
	+S	116,93	45,39		85,167	124.3 ± 20.4**
		Change in catecholamine release evoked by stimulation ($pmolmin^{-1}kg^{-1}$)				
Control	+S	75,87	14,13	49	83	99.8 ± 21.3
Captopril	+S	52,19	0,-2		34,71	44.9 ± 21.3*

+S = During splanchnic nerve stimulation (10pps,10v)
C = Control
Statistical significance (captopril data compared with control data and C data compared with +S data)-:

* = $p > 0.05$
** = $p > 0.02$
*** = $p > 0.001$

Figure 3·2

The effect of captopril on total catecholamine (CA) release, before and after splanchnic nerve stimulation, from the denervated adrenal gland

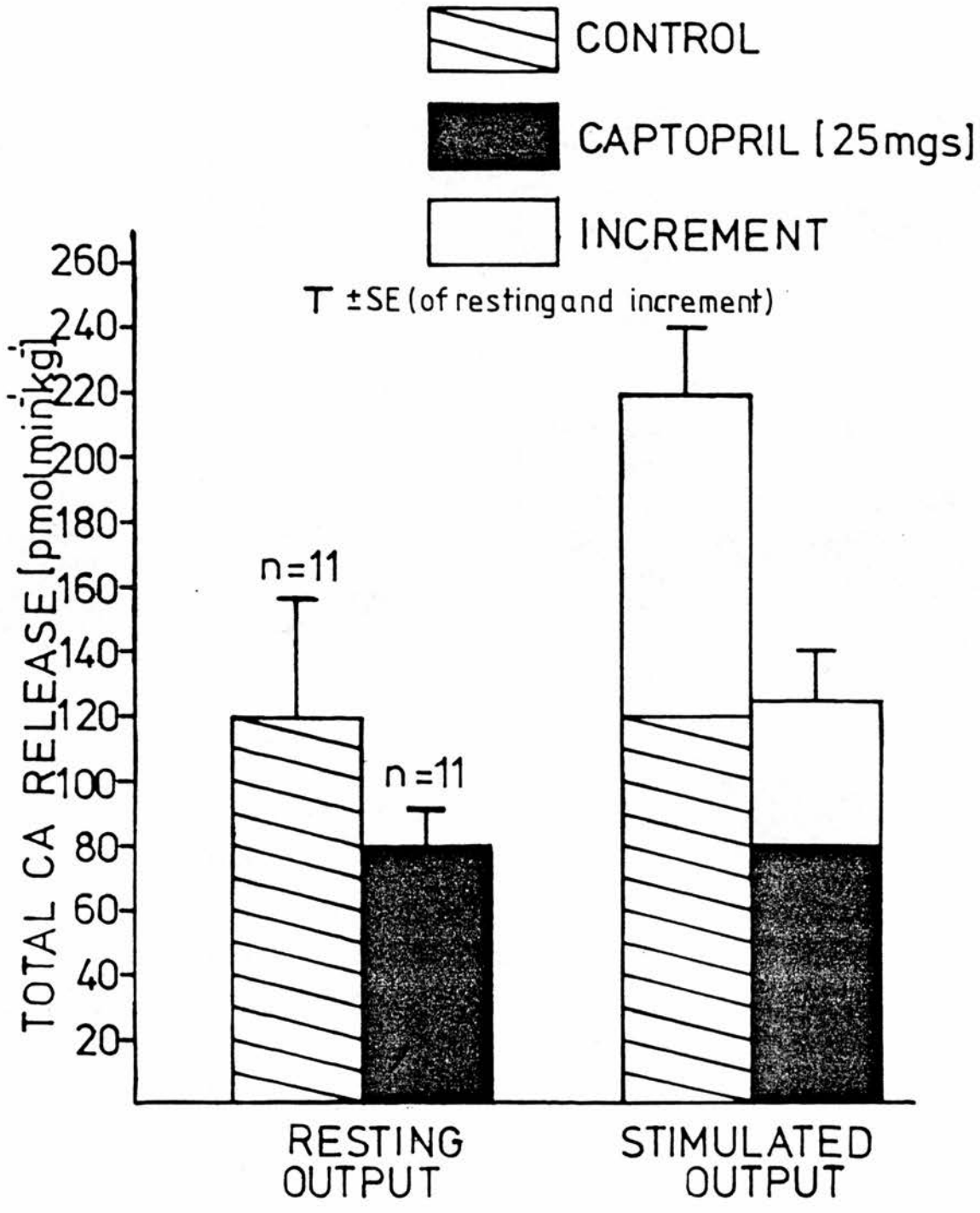


Table 3.4

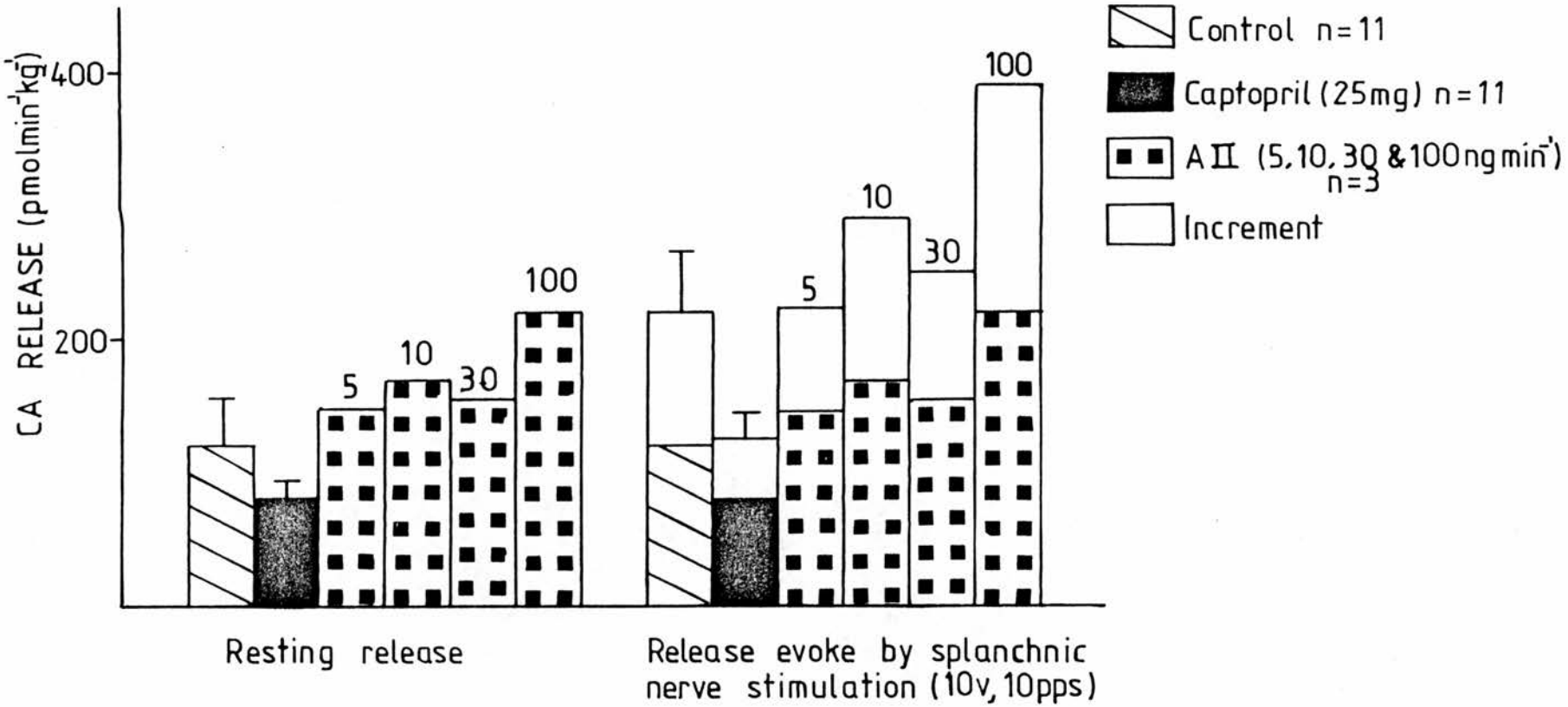
The effect of angiotensin II (AII) on adrenal catecholamine release before and after splanchnic nerve stimulation (10pps,10v), and following captopril administration, in dogs 26-28 (D26-28).

Drug Treatment		Total catecholamine release ($pmolmin^{-1}kg^{-1}$) D26	D27	D28	Mean ± SE (n = 3)
AII ($10ngmin^{-1}$)	C	64	302	137	167.7
	+S	206	489	169	288.0
AII ($30ngmin^{-1}$)	C	66	306	79	150.3
	+S	251	358	137	248.7
AII ($100ngmin^{-1}$)	C	104	472	87	221.0
	+S	271	750	147	389.3
		Change in catecholamine release induced by stimulation ($pmolmin^{-1}kg^{-1}$) D26	D27	D28	
AII ($10ngmin^{-1}$)	+S	142	187	32	76.3
AII ($30ngmin^{-1}$)	+S	185	52	58	120.3
AII ($100ngmin^{-1}$)	+S	167	278	60	168.3

+S = During splanchnic nerve stimulation (10pps,10v)
C = Control

Figure 3·3

The effect of angiotensin II (AII), following captopril administration, on adrenal catecholamine (CA) release before and after splanchnic nerve stimulation.



AII restored both the resting release and stimulated release of catecholamines. Figure 3.3 shows that for 5,10 and 100 $ngmin^{-1}$ infusions of AII, these effects tend towards being dose-dependant.

3. The effect of saralasin on adrenal catecholamine release, before and after splanchnic nerve stimulation

It was of interest to investigate the effect of the AII-antagonist saralasin on adrenal catecholamine release, to compare its effect with captopril and rule out the possibility that the effects of captopril were unrelated to its inhibition of the renin-AII system.

The effect of saralasin on both the resting release of catecholamines and the release induced by splanchnic nerve stimulation, was investigated in dogs 29-31. Control adrenal blood samples before and after splanchnic nerve stimulation were obtained, then a continuous infusion of $10\mu gmin^{-1}kg^{-1}$ saralasin was administered and the stimulation tests repeated 10 and 20 min. later. The infusion of saralasin was then stopped, and the stimulation tests repeated 25 minutes later.

The effect of saralasin on total catecholamine release, before and after splanchnic nerve stimulation was analysed. The effect of saralasin on the change in catecholamine release following splanchnic nerve stimulation was analysed. The results are shown in table 3.5. The results shown in table 3.5 are illustrated in figure 3.4.

Table 3.5

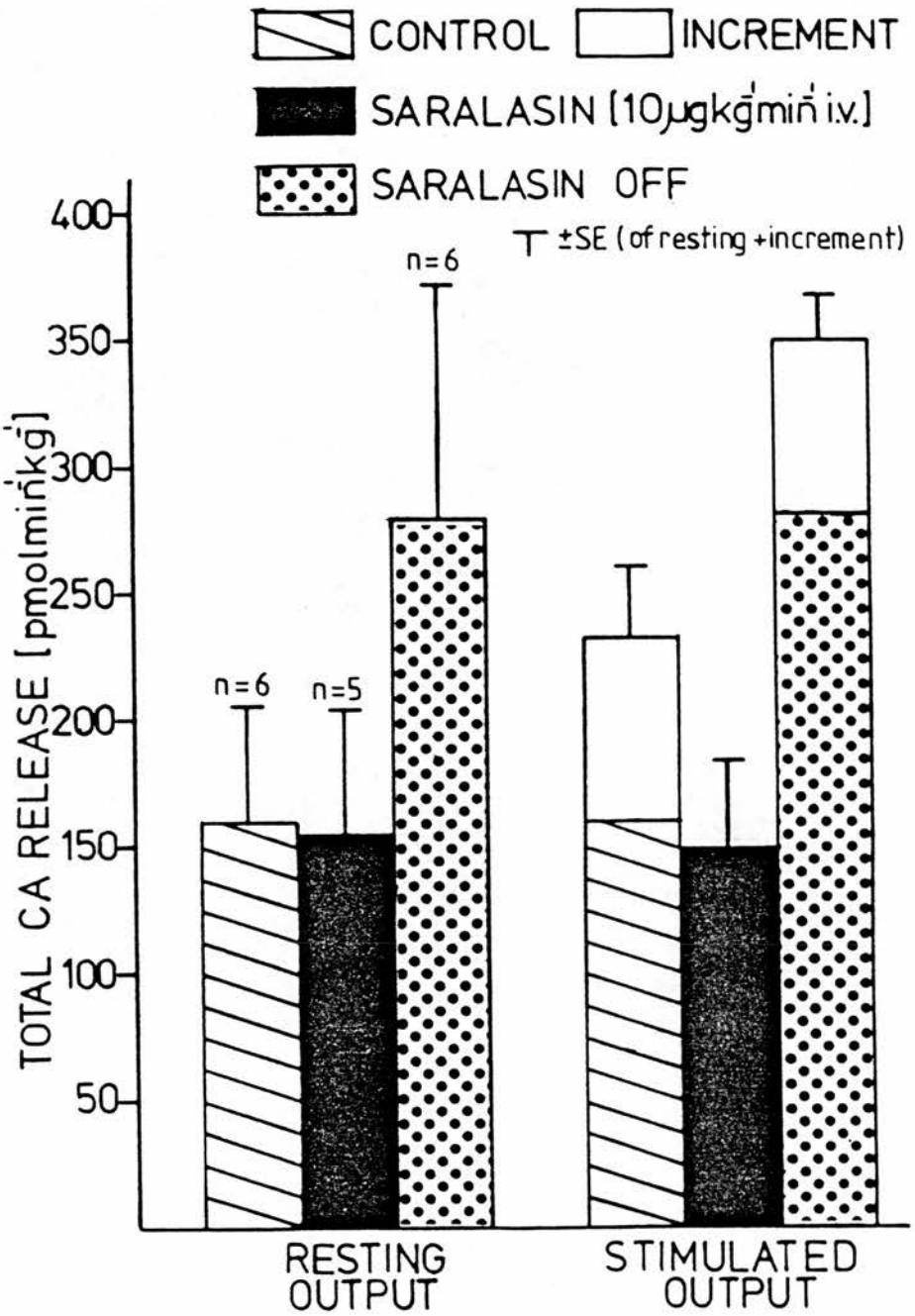
The effect of saralasin on adrenal catecholamine release before and after splanchnic nerve stimulation (10pps,10v) in dogs 29-31 (D29-31).

Drug Treatment		Total catecholamine release ($pmolmin^{-1}kg^{-1}$) D29	D30	D31	Mean ± SE
Control	C	52,58	67,282	272,226	159.5 ± 45.7
	+S	92,197	91,290	320,409	233.2 ± 52.7
Saralasin ($10\mu gmin^{-1}kg^{-1}$)	C	30,39	262	202,239	154.4 ± 49.9
	+S	49,67	133	260,236	149.0 ± 42.9*
Saralasin infusion off	C	54,56	213,619	282,458	280.3 ± 91.7
	+S	86,164	279,756	323,479	347.8 ± 98.6
		Change in catecholamine release induced by stimulation ($pmolmin^{-1}kg^{-1}$) D29	D30	D31	
Control	+S	40,139	24,8	48,183	73.7 ± 28.8
Saralasin ($10\mu gmin^{-1}kg^{-1}$)	+S	19,28	-129	58,-3	-5.4 ± 32.4**
Saralasin infusion off	+S	32,108	66,13 7	41,137	67.5 ± 18.8

+S = During splanchnic nerve stimulation (10pps,10v)
C = Control
Statistical significance (Saralasin data compared with control data)-:
* = $p < 0.02$
** = $p < 0.01$

Figure 3·4

The effect of saralasin on total catecholamine (CA) release, before and after splanchnic nerve stimulation, from the denervated adrenal gland



The results show that in these dogs, saralasin did not affect the resting release of catecholamines but inhibited the release of catecholamines evoked by splanchnic nerve stimulation. After the saralasin infusion was turned off, there was an increase in resting catecholamine output and the release of catecholamines evoked by splanchnic nerve stimulation was restored.

Comment

It may be that the large variations in catecholamine release seen between dogs, together with the limited sample number, obscured any effect that saralasin may have had on the resting release of catecholamines, as in dog 29, saralasin did reduce the resting output of catecholamines in both samples analysed (see table 3.5). This is also suggested by the increase in the resting release once the saralasin infusion was turned off. This type of overshoot usually occurs after a system has been depressed. The possibility that the lack of effect on resting output could have been due to some partial agonist activity of saralasin seems unlikely in view of the profound inhibitory effect on stimulated release.

4. The effect of captopril and the subsequent AII infusion on the blood pressure responses to splanchnic nerve stimulation

There was a small increase in systemic blood pressure (SBP) during splanchnic nerve stimulation. In the results section (10) of Part 1, I demonstrated how AII has a varying influence on the vascular resistance in different vascular beds. It was of interest to

investigate the effect of captopril and AII on the blood pressure responses to splanchnic nerve stimulation.

The effect of captopril and the subsequent AII infusion on the SBP responses induced by baroreceptor stimulation were analysed in dogs 19-28. The results are shown in table 3.6.

The results show that although captopril reduced the resting SBP, it had no effect on the SBP response to splanchnic nerve stimulation. This is a similar result to that demonstrated with the hind limb perfusion pressure response to baroreceptor stimulation. AII had no additional effect on either resting SBP or the SBP response to splanchnic nerve stimulation.

5. The effect of splanchnic nerve stimulation on adrenal blood flow

The effect of splanchnic nerve stimulation on adrenal blood flow, before and after captopril and a subsequent infusion of AII, was studied in dogs 19-28. The results are shown in table 3.7.

The results show that, unlike baroreceptor stimulation, splanchnic nerve stimulation did not induce an increase in adrenal blood flow. In these dogs, captopril and AII did not induce a significant effect on adrenal blood flow.

Table 3.6

The effect of captopril and the subsequent infusion of AII (AII) (~10ngmin^{-1}) on resting systemic blood pressure (SBP) and the increase in SBP induced by splanchnic nerve stimulation (SNS), in dogs 19-28.

Dog number	Resting SBP			SBP following SNS			Increment		
	Control	Captopril	AII	Control	Captopril	AII	Control	Captopril	AII
19	100	85	100	110	85	100	10	0	0
20	60	60	60	60	60	60	0	0	0
21	110	100	100	130	120	120	20	20	20
22	40	40	25	80	40	25	40	0	0
23	110	90	65	135	110	70	25	20	5
24	85	80	75	100	105	105	15	25	30
25	125,130	110,125		155,160	155,155		30,30	45,30	
26	120,115	85,100	115	135,120	95,110	115	15,5	10,10	0
27	90	70	70	90	70	70	0	0	0
28	105,105	95,75	75	115,115	105,85	90	10,10	10,10	15
Mean ± SE	99.6 ± 7.1 (n=13)	85.8* ± 6.1 (n=13)	76.1 ± 8.9 (n=9)	115.8 ± 7.9 (n=13)	99.6 ± 9.2 (n=13)	83.9 ± 10.2 (n=9)	16.2 ± 3.4 (n=13)	13.8 ± 3.8 (n=13)	7.8 ± 3.7 (n=9)

Statistical significance (captopril data compared with control data)-:
* = $p < 0.001$

Table 3.7

The effect of splanchnic nerve stimulation on adrenal blood flow, before and after administration of captopril and the subsequent infusion of AII (~10ngmin^{-1}), in dogs 19-28.

Dog number	Adrenal blood flow (mlmin^{-1})					
	Control		Captopril		AII	
	C	+S	C	+S	C	+S
19	6.4	7.0	2.7	2.7	3.2	3.4
20	2.5	2.5	3.3	3.1		
22	0.8	3.5	2.8	2.5	1.4	1.2
23	5.8	5.8	4.7	6.6	2.8	2.5
24	5.5	5.1	3.8	4.0	2.7	2.4
25	6.7	5.5	7.6	6.5	5.5	5.0
25	6.0	5.0				
26	2.4	2.2	2.7	2.1	3.8	4.3
26	2.5	2.5	3.5	3.6		
27	5.4	1.9	6.0	6.5	5.5	5.3
27	5.0	4.5	5.5	5.5		
28	7.0	6.2	8.7	8.2	9.5	9.9
28	6.2	5.7	10.2	11.0		
Mean ± SE	4.8 ± 0.6 (n=13)	4.4 ± 0.5 (n=13)	5.1 ± 0.7 (n=12)	5.2 ± 0.8 (n=12)	4.3 ± 0.9 (n=8)	4.3 ± 0.9 (n=8)

C = Control +S = During splanchnic nerve stimulation

Part 3 - Summary of results

1. Splanchnic nerve stimulation (10volts,2ms) in the denervated adrenal gland induced a release of catecholamines from the adrenal medulla. The release was frequency dependant between 2.5 and 10pps and maximal at 10pps.

2. Captopril (25mg) inhibited both the resting adrenal catecholamine release and the release evoked by splanchnic nerve stimulation.

3. Angiotensin II (5, 10 and 100ngmin^{-1}) reversed the inhibitory effect of captopril on both resting and stimulated adrenal catecholamine release, in a dose dependant manner.

4. Saralasin (10μgmin^{-1}kg^{-1}) inhibited adrenal release of catecholamines evoked by splanchnic nerve stimulation.

5. Captopril had no effect on the systemic blood pressure response to splanchnic nerve stimulation.

6. Splanchnic nerve stimulation did not induce an increase in adrenal blood flow.

Part 3 - Discussion of results

As has been discussed in "Part 1", Feuerstein etal (1977) proposed that the effect of angiotensin II (AII) on adrenal catecholamine release, following haemorrhage, was due to a central activation of sympathetic drive. The results of "Part 1" indicated that, alternatively, AII was exerting a direct effect on adrenal catecholamine release and that a minimum, non-pressor level of AII was required for the gland to respond to the reflex stimuli.

These results provide additional support for this conclusion. They show that in the denervated adrenal gland, captopril inhibited the resting adrenal catecholamine release, an effect which was reversed by a non-pressor level of AII. In this case, release cannot have been due to a central activation of sympathetic drive, as the gland was denervated, as described in the "Methods" section. This indicates that the effect of AII is a direct facilitatory one at the level of the adrenal gland. It supports the hypothesis that a minimum level of AII is required for the gland to respond fully to neural drive.

The results show that AII increased catecholamine output in a dose dependant manner for 5, 10 and 100ngmin^{-1}. In the "Introduction and literature review, the evidence supporting a role for AII in facilitating sympathetic neuro-effector transmission was discussed. It has been shown to facilitate noradrenaline release from other sympathetic ganglia (Reit, 1972; Starke, 1972) and to enhance stimulation-evoked acetylcholine overflow from parasympathetic

ganglia (Panisset, 1968).

The results described here suggest that AII has a facilitatory effect on adrenal catecholamine release evoked by splanchnic nerve stimulation. It was not possible to determine if there was a prejunctional effect on acetylcholine overflow in these experiments. As has been discussed previously, AII does facilitate adrenal catecholamine release by a direct effect on the adrenal gland and does depolarise chromaffin cells (Douglas etal, 1967), hence it does exert a "post-junctional" effect if the adrenal medulla is considered a specialised sympathetic ganglion. AII may facilitate stimulation-evoked acetylcholine release from the splanchnic nerve terminal in an analagous fashion to its effect on other nerve terminals, but this cannot be concluded from these experiments so this is a speculative suggestion. As was discussed in the "Introduction and literature review", most of the available evidence indicates that the most important facilitatory effect of AII on sympathetic nerve terminals is on stimulation-evoked release of noradrenaline. My results suggest that AII also facilitates basal catecholamine release from the adrenal medulla, as AII restored the resting release of catecholamines previously inhibited by captopril.

We stimulated the greater splanchnic nerve trunk, which has been shown to evoke an output of catecholamines twice that detected after stimulation of all remaining splanchnic nerve fibres in dogs (Douglas, 1975), and so were stimulating a very high proportion of the maximum amount of catecholamines possible. The most effective frequency of splanchnic nerve stimulation in most species lies within the range 15-

40pps (Douglas, 1975) and is frequency dependant. In these experiments, we observed a maximum stimulation between 10-20pps, and release was frequency dependant between 2.5 and 10pps.

The resting output of catecholamines from the denervated glands of these dogs was relatively high compared with those previously observed. Tables 3.1 and 3.2 show that before and after captopril administration, splanchnic nerve stimulation at 2.5pps failed to induce catecholamine release, in fact there was an inhibition of release. These results suggest that there may be an inhibitory component of splanchnic nerve stimulation, uncovered by denervation, and not overcome until the nerve was stimulated at 5.0pps. This is a tentative suggestion and it would require further investigation to verify this.

The AII antagonist, saralasin also inhibited the adrenal release of catecholamines evoked by splanchnic nerve stimulation, which confirms that the effect of captopril is mediated through an inhibitory effect on angiotensin converting enzyme.

Captopril did not affect the small systemic blood pressure response to splanchnic nerve stimulation. This indicates that as for the perfused hind limb vascular bed, AII has no effect on the vascular bed involved in the small blood pressure response to splanchnic nerve stimulation. It does, as described in "Part 1", affect the pressor response to baroreceptor stimulation which confirms that AII has varying influences on the vascular resistance of different vascular beds.

Unlike baroreceptor stimulation, splanchnic nerve stimulation did not increase adrenal blood flow. This is probably due to the great differences in the stimulation parameters involved during splanchnic nerve stimulation and baroreceptor stimulation, the latter evoking a more "physiological" stimulation of the adrenal gland. The direct stimulation of the splanchnic nerve may have involved a greater number of vasoconstrictor fibres, but the actual reason for this difference cannot be accurately determined, as it is not possible to compare accurately the stimulation parameters involved in this direct stimulation and those involved after an increase in total sympathetic tone following a decrease in carotid perfusion pressure.

The results show that despite denervation, there was still a considerable resting output of catecholamines from the adrenal medulla. Coupland (1975) described how resting output can be reduced when the splanchnic nerves are sectioned and is therefore partly due to ongoing secretomotor tone from the central nervous system. The remaining secretion is often resistant to cholinergic antagonists. It was not possible to measure catecholamine output before and after splanchnic nerve denervation in these experiments as this would have involved two stages of surgery which was not possible. In the denervated gland, resting output may be driven by humoral influences including steroids, AII and prostaglandins (see "Part 5"). This is supported by the results discussed in "Part 2", which demonstrated that even in the innervated gland, coadministration of captopril and cycloheximide severely inhibited the resting release of catecholamines.

In conclusion, the results suggest that a non-pressor circulating level of AII has a direct facilitatory effect on adrenal catecholamine release. This effect of AII does not depend on central activation of sympathetic drive. As with sympathetic nerves and other sympathetic ganglia, AII may facilitate splanchnic nerve activity.

Introduction to Parts 4 and 5

Having fulfilled the main objectives of this research project, I had only a limited ammount of research funding remaining. I had the choice of either investigating one more area of interest in detail or carrying out introductory experiments in two areas. I chose to do the latter as I was very interested in the possible interactions between both prostaglandins and endogenous opioid peptides and adrenal catecholamine release. It appeared possible that captopril may affect the synthesis of prostaglandins and the breakdown of endogenous opioid peptides, and this may contribute to some of the effects of captopril. The experiments described in parts 4 and 5 were therefore performed on limited numbers of dogs and must therefore be regarded as introductory experiments, additional to the main core of this research.

Part 4

Captopril has been shown to inhibit the action of the carboxypeptidase enzyme responsible for the metabolism of endogenous opioid peptides in vitro (Arregiu, Lee, Emson and Iversen, 1979), and also to reduce the metabolism and increase the analgesic potency of met-enkephalin (Stine, Yang and Costa,1979). Rubin, Millar, Sturani, Lawrie and Reid (1984) presented evidence which suggested that naloxone may attenuate some of the cardiovascular effects of captopril in man (see discussion).

Opioid peptides were first detected in the brain (Stjarne, 1973; Hughes, Smith, Kosterlitz, Fathergill, Morgan and Morris, 1975; Yang, Hong and Costa, 1977). I shall review the more recent evidence that demonstrates their presence in the splanchnic nerves and the adrenal medulla and that they exert an inhibitory effect on the release of catecholamines from the adrenal medulla.

It appears possible that captopril, by inhibiting the metabolism of the opioid peptides, may increase their inhibitory effects on the release of catecholamines from the adrenal medulla, and this could contribute to the inhibitory effects of captopril on the adrenal catecholamine release.

Schultzberg, Lundberg, Hokfelt, Terenius, Brandt, Elde and Goldstein (1978) demonstrated the presence of enkephalins in the superior cervical ganglion, inferior mesenteric ganglion and the coelic superior mesenteric ganglion of the guinea pig and rat, and

demonstrated their presence in the adrenal medulla of the rat, guinea pig and cat. These observations have subsequently been confirmed by others (Di Guilio, Yang, Lutold, Fratta, Hong and Costa, 1980; Lewis, Stern, Kimura, Rossier, Stein and Udenfriend, 1980). In addition to the enkephalins, dynorphine, a potent opioid heptadecapeptide which was first isolated from porcine pituitaries (Goldstein, Tachibana, Lowney, Hunkapillar and Hood, 1979), has been detected in the bovine adrenal medulla (Dumont, Day and Lemaire, 1983). It has been found to be the second most abundant opioid peptide in the adrenal medulla after leu-enkephalin and its secretion from isolated chromaffin cells has been demonstrated (Lemaire, Denis and Day, 1982; Denis, Day and Lemaire, 1982).

Most of the available evidence seems to indicate that the enkephalins are stored within adrenaline containing chromaffin cells and not costored with noradrenaline in the bovine adrenal medulla (Livett, Day, Elde and Howe, 1982; Pelto-Huikko, Salminen and Hervonen, 1982). Lang, Taugner, Gaida, Ganten, Kraft, Unger and Wunderlich (1983) demonstrated that bovine chromaffin medullary cells respond to nicotinic stimulation by releasing enkephalins and adrenaline in proportions similar to the cellular content of both whereas a much higher proportion of noradrenaline is released under these conditions (Livett, Dean, Whelan, Udenfriend and Rossier, 1981), indicating costorage of enkephalins mainly within adrenaline containing cells. Dynorphine has also been detected exclusively in bovine adrenaline containing chromaffin cells (Dumont etal, 1983). Schultzberg etal (1978) however, demonstrated that in the cat and guinea pig, enkephalin-like immunoreactivity was widespread and

evident in both noradrenaline and adrenaline containing cells, whereas in the rat only a few medullary cells showed enkephalin-like immunoreactivity, and these were noradrenaline containing cells. In rats, however there was evidence of enkephalins within adrenaline containing cells after denervation of the adrenal gland. The evidence suggests there may be species differences in the location of enkephalins within the adrenal medulla.

Enkephalins are also stored in the axon terminals of the splanchnic nerve (Schultzberg etal, 1978; Di Guilio, Yang, Fratta and Costa, 1979) and they are the most abundant of the neuro-peptides both in the adrenal medulla and the splanchnic nerves (Livett etal, 1982; Udenfriend and Kilpatrick, 1983; Viveros and Wilson, 1983). The inhibitory effects of morphine on the release of catecholamines from the adrenal medulla may be enhanced with stimulation of the splanchnic nerve (Anderson and Slotkin, 1976), and evidence indicates that the enkephalins stored in the splanchnic nerves may act as co-transmitters, decreasing the activity of nicotinic stimulation of the adrenal medulla by down regulating the nicotinic receptor sites. In addition opioid peptide levels in the adrenal medulla are positively related to the activity of the splanchnic nerves (Schultzberg etal, 1978).

Elliott (1912) first demonstrated that morphine affected the release of adrenaline from the adrenal medulla, but since then both in vitro and in vivo experiments indicate that opioid peptides inhibit the stimulated release of catecholamines from the adrenal medulla.

In vivo, administration of morphine decreases, and naloxone, an opiate antagonist, increases plasma catecholamine levels. Earlier studies using morphine, and other opiate agonists, led to controversial results, probably due to the dose and time related side effects elicited by such agonists (Borrell, Lorens and Borrell, 1974; Domino, Vasko and Wilson, 1976). For example, in dogs, morphine can be seen to increase, decrease or not affect plasma catecholamine levels depending on the dose and experimental conditions used (Taborsky, Halter and Porter, 1981). It has also been shown that in the cat, morphine has a biphasic effect on blood pressure, dependant on the dose used (Wallenstein, 1979). Such problems are not encountered with naloxone which is now more often used to study the effects of opioid peptides on plasma catecholamine levels.

Naloxone does not seem to modify the peripheral levels of noradrenaline in man (Data, Gerber, Crump, Frolich, Hollifield and Nies, 1978), but Mannelli, Maggi, De Feo, Cuomo, Delitala, Gusti and Serio (1984) have shown that in man, naloxone does cause an increase in plasma adrenaline levels fifteen minutes after administration, which indicates an effect on the adrenal medulla. This increase in plasma adrenaline was, although significant, not enough to modify the blood pressure and heart rate.

In vitro studies also suggest an inhibitory role for endogenous opiates on the release of catecholamines from the adrenal medulla.

Yoshizaki (1973) demonstrated that morphine could affect catecholamine release from denervated adrenal glands, indicating that

they can affect catecholamine release from the adrenal medulla directly, independant of splanchnic nerve activity. This was confirmed by Anderson and Slotkin (1976) who demonstrated that in both innervated and denervated adrenal glands, morphine induces an increase in catecholamine release. This would explain why morphine causes a depletion of catecholamines in the adrenal medulla of new- born rats even though splanchnic innervation was not yet functional (Anderson and Slotkin, 1975). They also demonstrated that the release of catecholamine was accompanied by a compensatory induction of tyrosine hydroxylase and dopamine hydroxylase. This effect on dopamine hydroxylase was unlikely to be due to ACTH as morphine treatment resulted in an increase in the enzyme activity as early as 24 hours after the first injection, an effect which cannot be duplicated by ACTH administration (Weinshilboum and Axelrod, 1970). As dopamine hydroxylase is a marker enzyme for storage vesicles, Anderson and Slotkin suggest that morphine induces an increase in storage vesicle synthesis.

Konishi, Tsunoo and Otsuka (1979) reported that enkephalins inhibited cholinergic transmission in the sympathetic ganglia of guinea pigs, and in 1980, Kumakura, Karoum, Guidotti and Costa demonstrated that nicotinic stimulation of catecholamine release from isolated bovine chromaffin cells could be reduced by leu- and met-enkephalin. The concentrations of the opioid peptides used were very high, however, and subsequent studies demonstrated that their effects could not be reversed by naloxone and showed no stereospecificity (Lemaire, Day, Dumont, Chovinard and Calvert, 1984). This suggests that inhibition of the nicotinic responses in vitro by leu- and met-

enkephalin are probably not mediated via the conventional high affinity stereospecific opiate receptors (Livett, Boska, Dean, Mizobe and Lindenbaum, 1983). Costa, Guidotti, Hanbauer and Saiani (1983) agree with this suggestion, noting that the opiate recognition sites of the adrenal medulla have a high affinity for met-enkephalin (which is stored in the splanchnic nerves). They confirmed that they cannot be classified with any one of the classical criteria which characterise opiate receptors by the narcotic agonists and/or antagonists that bind preferentially to the receptor (Snyder and Goodman, 1980).

Lemaire, Livett, Tseng, Mercier and Lemaire (1981) also demonstrated a dose-dependant inhibition, by opioid peptides, of the responses of isolated bovine chromaffin cells to acetylcholine. Again the concentrations involved were extremely high.

Costa etal (1983) reported evidence to show that the opiates not only act on acetylcholine-mediated catecholamine release from primary cultures of chromaffin cells, but also modulate the physiological release of catecholamines from the adrenal medulla in vivo. They demonstrated that in the dog, naloxone facilitated the release of opioid peptides and catecholamines from the adrenal medulla following splanchnic nerve stimulation. They had also previously shown that the content of met-enkephalin in the adrenal gland and other sympathetic ganglia is lower in spontaneously hypertensive rats (SHR) than normotensive rats (Di Guilio etal, 1979). The decrease in enkephalin mediated inhibition of catecholamine release from the adrenal medulla could contribute to the hypertension in SHR.

As has been discussed, AII facilitates the activity of the sympathetic nervous system and release of catecholamine from the adrenal medulla. The evidence discussed here suggests an opposite role for the opioid peptides which inhibit the release of catecholamines from the adrenal medulla. There has been little or no work done on any possible interaction between the activities of AII and opioid peptides, but as mentioned, captopril does inhibit the activity of carboxypeptidase and may induce an increase in either splanchnic nerve or adrenal medullary opioid peptides, and it is possible that this could contribute to its inhibitory effect on catecholamine release.

The aim of this series of experiments was to investigate:-

1. The effects of naloxone on the resting, and baroreceptor stimulation induced, release of catecholamines from the adrenal medulla.

2. If naloxone could reverse the inhibitory effects of captopril on catecholamine release.

3. The effect of naloxone on the adrenal release of catecholamines induced by splanchnic nerve stimulation.

Part 4 - Results

1. The effect of naloxone administration, following captopril administration on adrenal catecholamine release before and after baroreceptor stimulation

The effect of naloxone administration ($0.3mgmin^{-1}$, continuous infusion), following captopril administration, on adrenal catecholamine release before and after six minute baroreceptor stimulation was studied in dog 32. The effect of naloxone on total catecholamine release, and the release induced by baroreceptor stimulation was analysed. The results are shown in table 4.1. The effect of captopril and naloxone on resting release of catecholamines, and the release 1-2 minutes after the onset of baroreceptor stimulation is illustrated in figure 4.1.

The results show that captopril inhibited and naloxone restored both the resting release of catecholamines and also the reflex release induced by baroreceptor stimulation.

2. The effect of naloxone administration, before and after captopril administration, on adrenal release of catecholamines, before and after baroreceptor stimulation

In dogs 33-35 the following experimental proce dure was adopted-: Control six minute baroreceptor tests were performed, then the naloxone infusion was started and the baroreceptor tests repeated 15 minutes later. The naloxone infusion was stopped and the baroreceptor

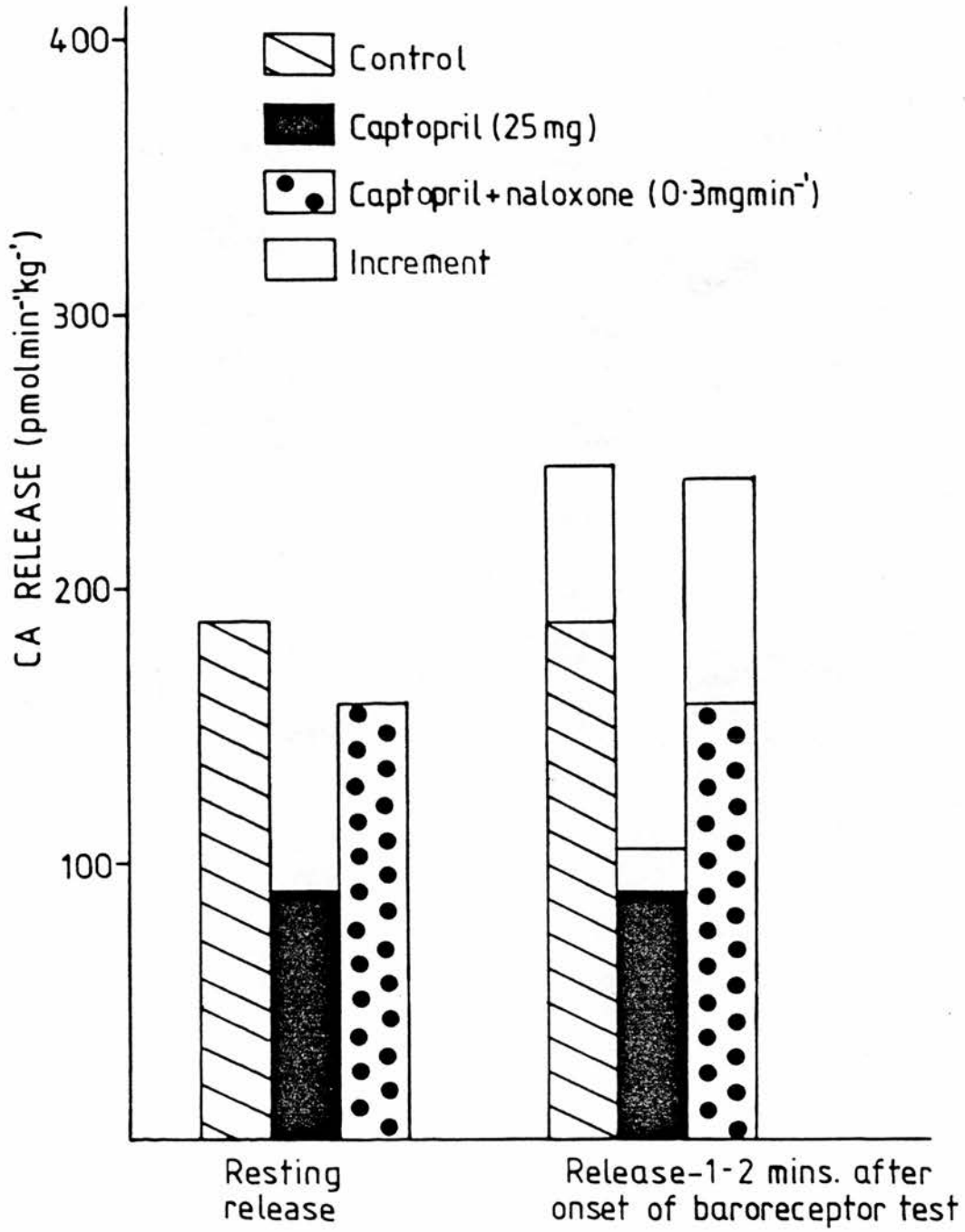
Table 4.1

The effect of naloxone administration, following captopril administration, on adrenal release of catecholamines before and after baroreceptor stimulation (BRS), in dog 32

Drug Treatment	Time after onset of BRS	Total catecholamine release ($pmolmin^{-1}kg^{-1}$)	Increment
Control	0	187	
	1-2	245	58
	5-6	240	53
Captopril (25mg)	0	90	
	1-2	100	16
	5-6	90	0
Naloxone ($0.3mgmin^{-1}$)	0	157	
	1-2	241	84
	5-6	355	198

Figure 4·1

Effect of naloxone on catecholamine (CA) release, following captopril administration, in dog 32.



tests repeated 30 minutes later. Captopril was administered and the tests repeated. The naloxone infusion was restarted, the tests repeated, then stopped once more and the tests repeated.

The effect of naloxone, before and after captopril administration and before and after baroreceptor stimulation, on resting catecholamine release and the change in release induced by baroreceptor stimulation was studied in dogs 33-35. The results are shown in table 4.2 and illustrated in figure 4.2.

Comments

The sample numbers in this study were very small and so only limited statistical evaluation was possible. They show that prior to captopril administration, naloxone alone induced an increase in resting adrenal catecholamine release (figure 4.2a). Turning off the naloxone infusion did not change the resting release but this was reduced by the subsequent administration of captopril. It should be noted that captopril did not reduce resting catecholamine release below the control values. Turning the infusion of naloxone on again did not restore the resting release of catecholamines, and the effect of turning the naloxone infusion back off was an increase in resting levels.

Due to the small sample numbers and the large variations in catecholamine release between dogs, I can only infer that naloxone has a facilitatory effect on resting catecholamine release. Subsequent captopril administration resulted in a decrease in resting

Table 4.2

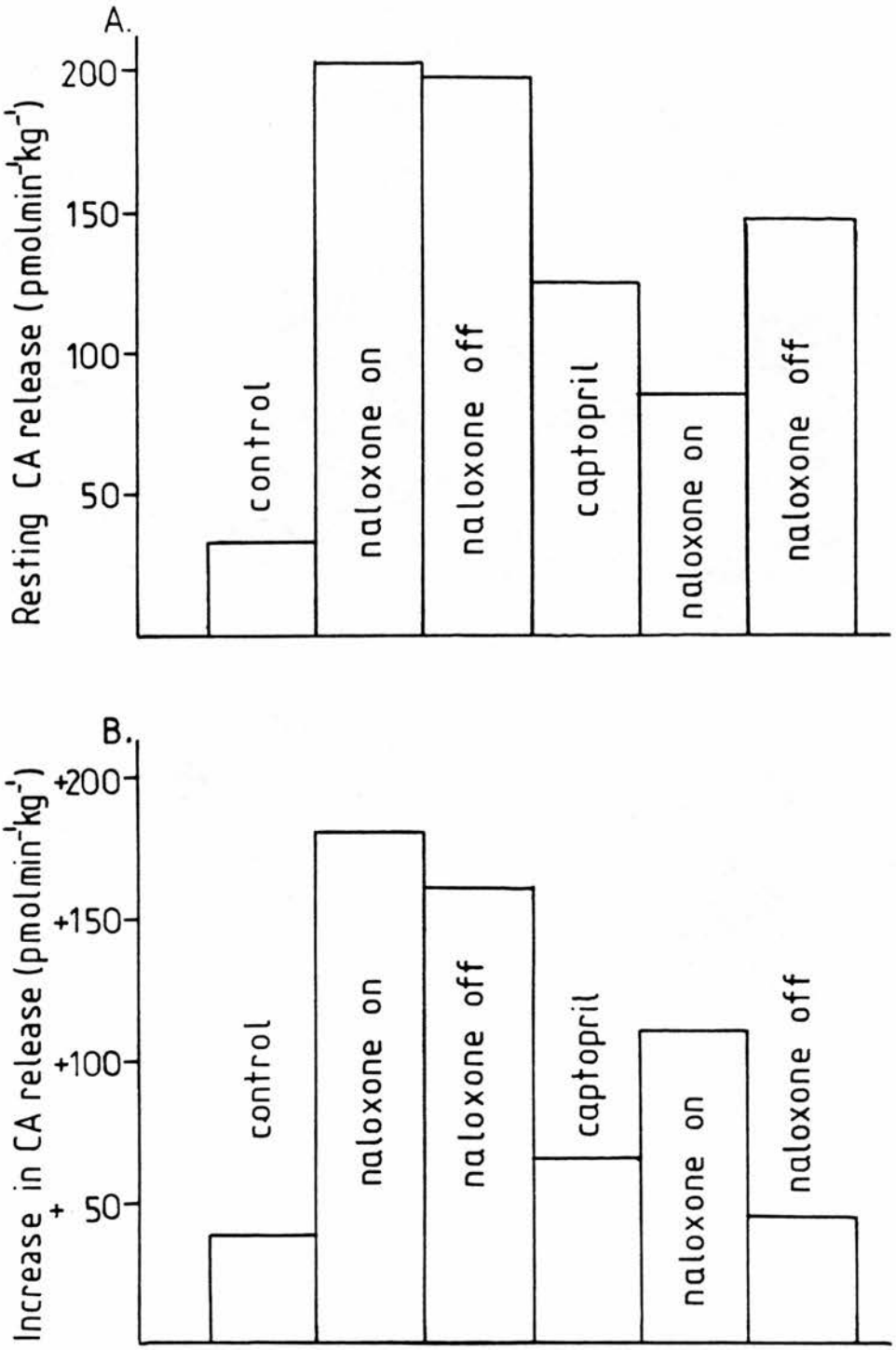
The effect of turning on and off an infusion of naloxone, before and after captopril administration, on resting catecholamine release and the change in cateholamine (CA) release induced by baroreceptor stimulation (BRS)

Drug Treatment	Time after onset of BRS (min)	Dog 33 Total CA	Dog 33 Inc	Dog 34 Total CA	Dog 34 Inc	Dog35 Total CA	Dog35 Inc	Mean Total CA ± SE	Mean Inc ± SE
Control	0	17		8,68		20,34		29.4 ± 10.5	
	1-2	34	17	34,144	26,27	54,140	34,106	81.2 ± 25.0	42.0 ± 16.2
	5-6	49	32	39	31	25,101	5,67	53.5 ± 16.6	33.8 ± 12.7
Naloxone on ($0.3mgmin^{-1}$)	0	137		393		77		202.3 ± 116.8	
	1-2	278	141	499	106	370	293	382.3 ± 64.1**	180.0 ± 57.4*
	5-6	231	94	494	101	146	69	290.3 ± 104.8	88.0 ± 9.7
Naloxone off	0	59		334				196.5	
	1-2	253	194	462	128			357.5	161.0
	5-6	135	76	493	159			314.0	117.5
Captopril (25mg)	0	82		202		89		124.3 ± 38.9	
	1-2	96	14	276	74	196	107	189.3 ± 52.1	65.0 ± 27.2
	5-6	143	61	290	88	132	43	188.3 ± 50.9	64.0 ± 13.1
Naloxone on	0	74		114		64		84.0 ± 15.3	
	1-2	151	77	310	196	122	58	194.3 ± 58.4	110.3 ± 43.2
	5-6	135	61	131	17	70	6	112.0 ± 21.0	28.0 ± 16.8
Naloxone off	0	75		261		107		147.7 ± 57.4	
	1-2	125	50	323	62	132	25	193.3 ± 64.9	45.7 ± 10.9
	5-6	84	9	281	20	127	20	164.0 ± 59.8	16.3 ± 3.7

Total CA = Total catecholamine release ($pmolmin^{-1}kg^{-1}$)
Inc = Increment in release from release 0 min after BRS
Statistal significance (naloxone data compared with control data)-:
* = $p > 0.02$
** = $p > 0.01$

Figure 4·2

The effect of turning on and off an infusion of naloxone (0·3mgmin^{-1}) before and after captopril (25mg) administration, on resting catecholamine (CA) release [A.] and the increase in the release induced 1-2 minutes after the onset of baroreceptor stimulation [B.].



(Figure compiled from data contained in table 4·2)

catecholamine release. This is compatible with parallel but opposite effects of naloxone and captopril on resting adrenal catecholamine release.

The results also show (figure 4.2b) that prior to captopril administration, naloxone alone induced an increase in the release of catecholamines induced by baroreceptor stimulation. Turning off the naloxone infusion did not change this reflex release but this was reduced by captopril administration, although not to below control values. Turning the infusion of naloxone back on again tended to reverse, although not completely the effect of captopril. Turning the infusion back off reverses the effect of the previous naloxone infusion.

Again, I can only infer that naloxone has a facilitatory effect on the reflex release of catecholamines, before and after captopril administration. In the light of the observation that after the initial infusion of naloxone, captopril did not reduce either resting or reflex release of catecholamines below control values, it is possible that a residual effect of naloxone remained, even though the infusion of naloxone had been turned off. These results are compatible with parallel but opposite effects of naloxone and captopril on the reflex release of catecholamines.

3. The effect of naloxone, before and after captopril administration on the release of catecholamines evoked by splanchnic nerve stimulation

The effect of naloxone administration before captopril administration was studied in dog 36. A control stimulation test was performed, then an infusion of naloxone was started and the tests repeated 15 minutes later. The naloxone infusion was switched off, and 20 minutes later captopril was administered and the tests repeated 20 minutes later. The same proceedure was carried out in dog 37, except that the naloxone infusion was turned back on again and the tests repeated.

The effect of naloxone on resting adrenal catecholamine release and the release induced by splanchnic nerve stimulation (10pps,10v) was analysed. The results are shown in table 4.3 and the results for dog 37 illustrated in figure 4.3 (the two values for each drug treatment were meaned).

The results show that naloxone induced an increase in both the resting and stimulation induced release of catecholamines. Captopril inhibited both the resting and stimulation induced release, and the subsequent infusion of naloxone reversed these effects of captopril. It should be noted, that as in the previous set of experiments, following the initial infusion of naloxone, captopril did not reduce resting catecholamine release below control values. It is again possible that there was some residual effect of naloxone remaining even though the naloxone infusion had been turned off. These results

Table 4.3

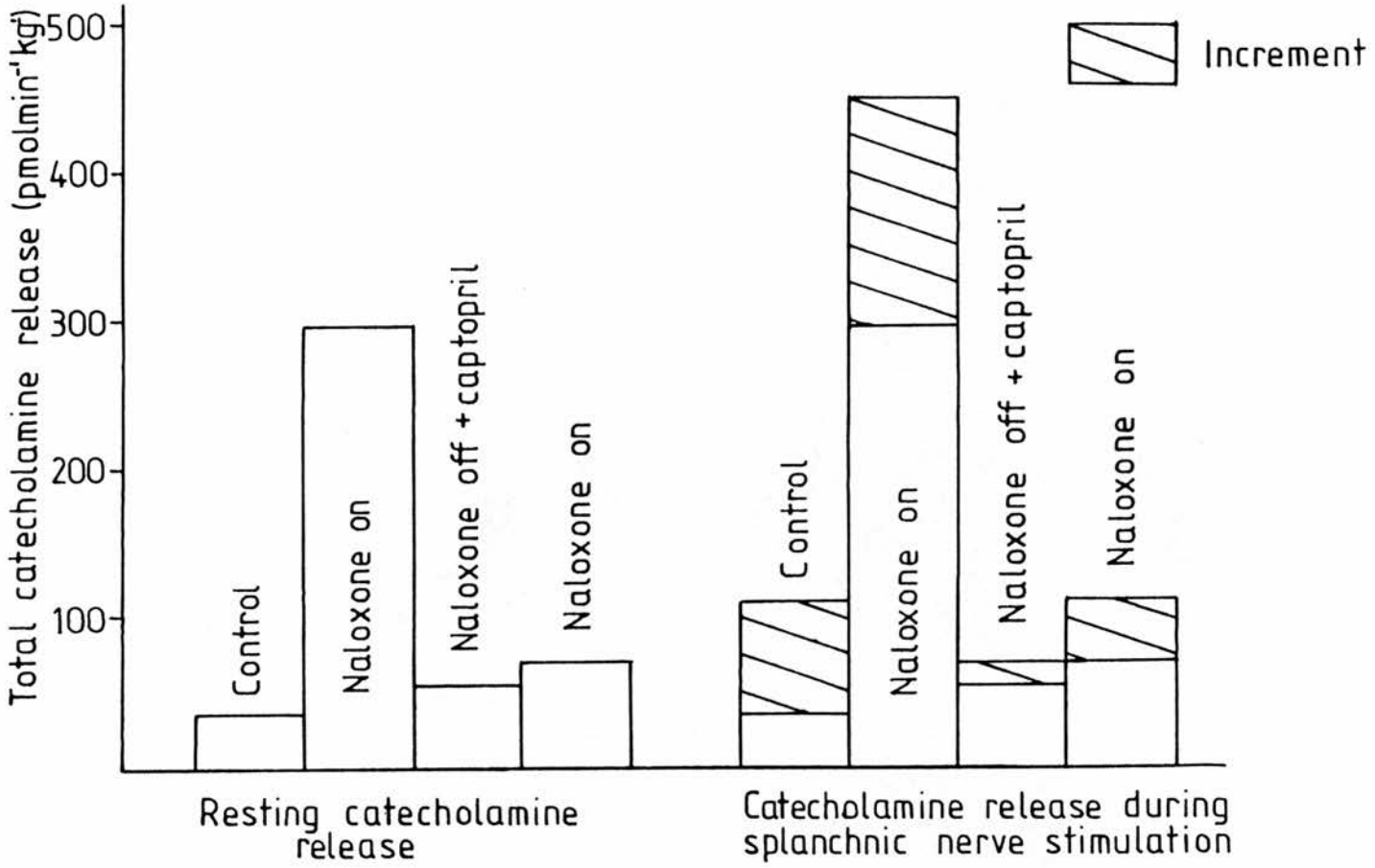
The effect of naloxone before and after captopril administration on adrenal catecholamine (CA) release, before and after splanchnic nerve stimulation, in dogs 36 and 37 (D36 and D37)

Drug Treatment	Function	Total CA release ($pmol min^{-1} kg^{-1}$) D36	D37	Increment D36	D37	Mean release ± SE	Mean Increment ± SE
Control	C	2	45,26	5	51,94	24.3 ± 12.4	50.0 ± 25.7
	+S	7	96,120			74.3 ± 34.4	
Naloxone on ($0.3 mg min^{-1}$)	C	6,10	187,406	22,14 1	127,178	152.3 ± 94.5	117.0 ± 38.6
	+S	28,151	314,584			269.3 ± 120.2	
Naloxone off + Captopril	C	34,43	49,61	-2,-22	21,6	46.8 ± 5.7	0.8 ± 9.0
	+S	32,21	70,67			47.5 ± 12.3	
Naloxone on	C		72			72	41
	+S		113			113	

C = control
+S = during splanchnic nerve stimulation (10v,10pps)

Figure 4·3

The effect of naloxone, before and after captopril administration, on adrenal catecholamine release, before and after splanchnic nerve stimulation.



are compatible with parallel but opposite effects of naloxone and captopril on the release of catecholamines from the denervated adrenal gland.

4. The effect of naloxone on systemic blood pressure, before and after captopril administration

Rubin etal (1984) suggested that co-administration of naloxone may attenuate the effects of captopril on blood pressure in man. It was therefore of interest to study the effect of naloxone on systemic blood pressure (SBP) before and after captopril administration in the anaesthetised dog.

The effect of naloxone on resting SBP, before and after captopril administration was studied in dogs 33,34,36 and 37. The results are shown in table 4.4.

The results show that naloxone had little effect on resting SBP prior to captopril administration, and when administered after captopril, naloxone did not reverse the hypotensive effect of captopril.

5. The effect of dietary sodium on the inhibitory effects of captopril on adrenal catecholamine release and systemic blood pressure

It has been discussed in "Part 1 - Introduction and literature review", that in man, the hypotensive effect of captopril is not

Table 4.4

The effect of turning an infusion of naloxone ($0.3 mg min^{-1}$) on and off, before and after captopril administration, on resting systemic blood pressure (SBP)

Drug Treatment	Resting SBP				Mean resting SBP ± SE
	Dog 33	Dog 34	Dog 36	Dog 37	
Control	140,95	65,60	125,115	115,115	103.8 ± 10.0
Naloxone on	90	100	140,135	120,100	114.2 ± 8.4
Naloxone off + Captopril	45	70	100,100	75,70	76.7 ± 8.5*
Naloxone on	35	70		70,70	61.3 ± 8.8

Statistical significance (Captopril data vs previous naloxone data)-:
* = $p > 0.001$

always related to baseline plasma renin activity (PRA). It was therefore of interest to investigate the effect of baseline PRA on the inhibitory effects of captopril in the anaesthetised dog.

5.a. The effect dietary sodium on plasma renin activity

Dogs 33,38 and 39 were fed with a salt free diet for one week prior to each experiment day. The daily salt free diet consisted of-: Tripe (100g boiled twice in tap water and once in distilled water) to which was added 80g caloreen (a glucose polymer), 30g casilan (instant milk protein) and 50g sago (boiled in distilled water). Each dog also received 300ml milk to which was added 50ml prosparol emulsion (arachis oil).

Dogs 32,34 and 40 were fed on a high sodium diet for two days prior to each experiment day. The daily diet consisted of a can of "Pedigree Chum - Beef and Heart" which was found to contain the highest sodium content in the "Pedigree" range, fresh liver (cooked in salted water) and salt tablets with milk.

The effects of these diets on PRA was analysed, and the results are shown in table 4.5.

The results show that PRA in the salt depleted dogs was higher than in salt loaded dogs. This was expected as low sodium intake is a stimulus for increased renin secretion. Captopril increased PRA as has previously been discussed (Part 1), though it increased PRA more in the salt depleted dogs. After captopril administration, PRA in the

Table 4.5

The effect of salt loading and salt depleting on plasma renin activity (PRA) ($ngAIml^{-1}hr^{-1}$), before and after administration of captopril

Control

	Salt depleted			Mean ± SE (n=6)	Salt loaded			Mean ± SE (n=6)
	Dog 33	Dog 38	Dog 39		Dog 32	Dog 34	Dog 40	
PRA	20.6	23.7	41.5	26.4	24.3	14.3	1.9	12.5
	10.8	20.7	40.9	± 5.0	20.3	10.0	4.0	± 3.6

After captopril

	Dog 33	Dog 38	Dog 39		Dog 32	Dog 34	Dog 40	
PRA	21.1	85.8	45.3	47.0	12.6	39.4	16.0	17.8
	24.6	78.9	26.3	± 11.7	7.0	20.0	11.7	± 4.7

Salt depleted = Fed on a salt free diet
Salt loaded = Fed on a high salt diet

salt loaded dogs remained lower than in the salt depleted dogs.

5.b. The effect of dietary sodium on the inhibitory effect of captopril on adrenal catecholamine release

The effect of dietary sodium on the inhibitory effect of captopril on adrenal catecholamine release, before and after six minute baroreceptor stimulation, was analysed in dogs 32-34, 38 and 39 (the catecholamine assay in dog 40 was unsuccessfull). The results are shown in table 4.6.

Comment

These results were difficult to analyse due to the small groups of values involved, and to the fact that in dog 34, captopril had been administered after naloxone and there was obviously a residual effect of naloxone, on catecholamine release, remaining. The resting catecholamine output also varied between dogs. If the changes in catecholamine output induced by baroreceptor stimulation are considered alone, however, from the results it can be seen that captopril reduced catecholamine release by approximately 101pmolmin^{-1}kg^{-1} and 44.6pmolmin^{-1}kg^{-1}, 1-2 and 5-6 minutes after baroreceptor stimulation respectively, in the salt depleted dogs. It can be seen that captopril reduced catecholamine release by approximately 23pmolmin^{-1}kg^{-1} in the salt loaded dogs, 1-2 minutes after baroreceptor stimulation. This is expected as the effect of captopril is mainly due to its inhibition of AII synthesis, and it would be expected to be more effective when PRA is elevated, as in the salt depleted dogs. These results could,

Table 4.6

The effect of salt loading and salt depleting on catecholamine output before and after baroreceptor stimulation (BRS), before and after captopril administration

Drug Treatment	Time after BRS (min)	Total catecholamine output ($pmol\,min^{-1}\,kg^{-1}$)						
		Salt depleted (high PRA)			Mean	Salt loaded (low PRA)		Mean ± SE
		Dog 33	Dog 38	Dog 39		Dog 32	Dog 34	
Control	0	17	5	96	39.3	187,171	8,68	108.5 ± 42.6
	1-2	34	33	234	100.3	245,303	34,144	181.8 ± 59.13
	5-6	49	16	178	81.0		39	
Captopril	0	82*	4	499	195.0	90,76	(202)*	83.0
	1-2	96	20	1081	399.0	106,160	(276)	133.0
	5-6	143	14	292	149.7	90,105	(290)	97.5
		Change in catecholamine output during BRS						
Control	0							
	1-2	17	28	637	227.3	58,132	26,76	73.0 ± 22.2
	5-6	32	11	195	79.3		31	
Captopril	0							
	1-2	14	16	349	126.3	16,84	(74)	50
	5-6	61	10	33	34.7		(88)	

* These results are affected by the fact that these dogs had previously received naloxone infusions. The result in dog 34 was omitted from consideration as there was evidence of a residual effect of naloxone.

Salt depleted = Fed on a salt free diet
Salt loaded = Fed on a high salt diet
PRA = Plasma renin activity

however, be overinterpretated due to the small sample numbers, especially of the salt loaded dogs. I therefore would not like to suggest that these results gave an accurate assessment of the effect of dietary sodium on the ability of captopril to reduce catecholamine release.

5.c. The effect of dietary sodium on the hypotensive action of captopril

The effect of dietary sodium on the effect of captopril on resting systemic blood pressure (SBP) was analysed in dogs 32-34,38 and 39. The results are shown in table 4.7.

Once again, for the reasons given previously I would not like to suggest that these results give an accurate assessment of the effects of dietary sodium on the hypotensive effect of captopril. The results do suggest that resting SBP is lower in salt loaded dogs than in salt depleted dogs. It should be noted, however, that even in the low renin state, captopril still exerted a profound effect on both SBP and adrenal catecholamine release, indicating that an elevated PRA state is not required for these effects of captopril (see discussion).

Table 4.7

The effect of salt loading and salt depleting on the hypotensive action of captopril

	Salt depleted			Mean ± SE	Salt loaded			Mean ± SE
	Dog 33	Dog 38	Dog 39		Dog 32	Dog 34	Dog 40	
SBP	140, 95	90, 80	110, 95	101.7 ± 8.6	85, 70	65, 60	120, 105	84.2 ± 9.8
SBB after captopril	45	85, 75	60	66.3 ± 8.8	65, 60	70	100, 90	77.0 ± 7.7

Salt depleted = Fed on a salt free diet (high plasma renin activity)
Salt loaded = Fed on a high salt diet (low plasma renin activity)

Part 4 - Summary of results

1. Naloxone ($0.3 mg min^{-1}$) administered before captopril increased both the resting release of catecholamines and the reflex release in response to baroreceptor stimulation. Administered after captopril, naloxone restored the reflex release of catecholamines inhibited by captopril.

2. Naloxone, administered before and after captopril, increased the resting release of catecholamines and the release evoked by splanchnic nerve stimulation, from the denervated gland.

3. Naloxone, administered before or after captopril, did not affect resting systemic blood pressure.

4. Plasma renin activity (PRA) was higher in dogs fed on a low salt diet than those fed on a salt loaded diet. There was some indication that the inhibitory effect of captopril on adrenal catecholamine release and systemic blood pressure was slightly greater in the high PRA state. The effect of captopril in the low PRA state were profound, however, indicating the inhibitory effects of captopril are not dependant on elevated PRA, although this may potentiate the effects.

Part 4 - Discussion of results

As discussed in the "Introduction and literature review", there is much evidence demonstrating the presence of opioid peptides in the adrenal medulla and splanchnic nerve terminals. Also discussed was the evidence indicating that opioid peptides inhibit cholinergic transmission in sympathetic ganglia, acetylcholine mediated catecholamine release from chromaffin cells and catecholamine release from the intact adrenal gland. In addition, Chaminade, Foutz and Rossier (1984) have demonstrated that most of the enkephalin-like material is in the form of large enkephalin containing peptides, particularly proenkephalin. They demonstrated that splanchnic nerve stimulation induced a release of met-enkephalin and proenkephalin that paralleled the output of catecholamines.

The evidence suggests that opioid peptides, released from chromaffin cells along with catecholamines, exert an inhibitory influence on adrenal catecholamine release. I could find little reference to work investigating the effect of naloxone on adrenal catecholamine release in the anaesthetised dog. The only corroborative evidence I found was by Costa etal (1983) who demonstrated that in the dog, naloxone facilitated the release of opioid peptides and catecholamines from the adrenal medulla, following splanchnic nerve stimulation. They also demonstrated that in the dog, during splanchnic nerve stimulation, as with catecholamine release, adrenal enkephalin release increased in a stimulus dependant fashion. They observed release when the splanchnic nerve was stimulated at 9Hz and 10 volts, similar parameters to those

used in my experiments.

The results show that naloxone increased resting, baroreceptor reflex- and splanchnic nerve-stimulated catecholamine release in the anaesthetised dog. When administered after captopril, this effect of naloxone resulted in a reversal of the inhibitory effect of captopril on adrenal catecholamine release.

Captopril has been shown to inhibit the carboxypeptidase enzyme or "enkephalinase" responsible for the breakdown of endogenous opioid peptides (Arregiu etal, 1979). This would result in an increase in endogenous opioid peptide levels. Swerts, Perdrisot, Malfroy and Schwartz (1979) investigated whether or not enkephalinase was identical with the angiotensin-converting enzyme (ACE). They reported that the same enzyme is probably responsible for the cleavage of enkephalins in striatal and lung preparations and the striatal enkephalinase exhibits characteristics analagous to those of purified ACE. Captopril did inhibit both enkephalinase and ACE, though it was more potent at inhibiting ACE. This indicates that while differences do exist between the two enzymes, captopril does inhibit the enkephalinase and it is possible this could contribute to its effects.

Rubin etal (1984) proposed that, if captopril were to act partially through an effect on endogenous opiates, then naloxone may attenuate the hypotensive effect of captopril. Although their results were inconclusive, they reported that there was an indication that coadministration of naloxone did attenuate the effect of captopril on blood pressure in human volunteers. They suggested that part of the

effect of captopril may be related to its ability to inhibit enkephalinase.

My results show that naloxone on its own increased resting, reflex and stimulated release of catecholamines, effects which opposed those of captopril. It would be easy to misinterpret results which indicate that, when administered after captopril, naloxone reversed the effects of captopril, to mean that this was due to the effect of captopril on enkephalinase. In the case of effects on the adrenal medulla, this can be explained by parallel but opposite effects of naloxone and captopril on adrenal catecholamine release.

The results support the additional evidence which suggests that endogenous opioid peptides, released along with catecholamines from the adrenal medulla and from the splanchnic nerve, inhibit resting adrenal catecholamine release and release evoked by baroreceptor stimulation and splanchnic nerve stimulation, from both innervated and denervated adrenal glands, respectively.

The results do not rule out the possibility, however, that part of the inhibitory effect of captopril on adrenal catecholamine release may be mediated through an inhibitory effect on enkephalinase.

The results show that, in the anaesthetised dog, naloxone administration, before or after captopril, did not affect resting systemic blood pressure and did not attenuate the hypotensive effect of captopril. This result contradicts those of Rubin etal (1984) which may be due to species differences. Rubin etal (1984) also investigated

whether or not naloxone could unmask a tachycardia in response to the hypotensive effect of captopril. Opioid receptor agonists had been shown to lower blood pressure without producing a compensatory tachycardia in animals and man (Rubin, MacLean and Reid, 1983). The hypotensive effect of captopril is not accompanied by a reflex tachycardia, which is consistent with a decrease in baroreceptor reflex activity (Shepherd, Campbell and Reid, 1982). If captopril were to act through an effect on endogenous opiates then, it was proposed, coadministration of naloxone may unmask a tachycardia. Rubin etal reported, however, that this did not occur. This cardiovascular effect of captopril was not therefore related to its ability to inhibit enkephalinase.

In conclusion, in the anaesthetised dog, naloxone increases resting adrenal catecholamine release and the release evoked by both baroreceptor stimulation and splanchnic nerve stimulation, from both innervated and denervated glands, respectively. These effects are opposite to those of captopril. The results suggest that endogenous opioid peptides, stored in the chromaffin cells of the adrenal medulla and in splanchnic nerve terminals and released with catecholamines, exert a tonic inhibitory effect on resting adrenal catecholamine release. Endogenous opiates, released from the adrenal medulla with catecholamines following baroreceptor stimulation or splanchnic nerve stimulation may exert a negative feedback on catecholamine release. The catecholamines stored in splanchnic nerve terminals and released with noradrenaline, may decrease nicotinic stimulation of the adrenal medulla. AII and endogenous opioid peptides therefore exert opposing effects on adrenal catecholamine release and this may provide a "fine

tuning" of adrenal catecholamine release.

Captopril may increase adrenal and splanchnic nerve endogenous opioid peptide levels which may contribute to its inhibitory effect on adrenal catecholamine release, but this remains purely speculative. The results do not indicate that, in the anaesthetised dog, this effect of captopril contributes to its hypotensive action.

Salt depletion has been shown to activate the renin-AII system in man (Haber, 1976; Conway, Hatton, Keddie and Davies, 1979; Niarchos, Pickering, Case, Sullivan and Laragh, 1979) and in the dog (Kawamura etal, 1984). It was of interest to investigate if the effects of captopril were related to PRA. The results show that salt depletion did increase PRA in the dogs studied when compared with the PRA of dogs fed on a high salt diet. These results were difficult to analyse for the reasons stated in the "Results, 5.b.". Although inconclusive, however, the results did suggest that captopril had a slightly greater inhibitory effect on adrenal catecholamine release and a greater hypotensive effect in the salt depleted, high PRA state. The results show, however, that captopril still exerts a considerable inhibitory effect on both adrenal catecholamine release and systemic blood pressure in the low PRA state. This shows that an elevated AII level is not a requirement for the effectiveness of captopril as was discussed in "Part 1 - Introduction and literature review". This is consistent with the hypothesis that it is a minimum circulating level of AII which is important for the response of the adrenal gland to reflex stimuli and splanchnic nerve activity. It may also suggest that

some "non-renin" related effects of captopril may exist, such as an effect on endogenous opioid peptides or prostaglandin synthesis (discussed in detail in "Part 5").

Part 5

Part 5 - Introduction and literature review

There is evidence to suggest that the "non-renin" mediated antihypertensive effect of captopril could be due to the ability of captopril to increase the synthesis of vasodilating prostaglandins.

The evidence supporting this theory is very strong, but as I read around the literature it became obvious that the actions of prostaglandins on sympathetic nerve activity, adrenal medullary secretion of catecholamines and renal renin release are very complex and the available evidence is often very contradictory. I shall therefore discuss the evidence in some detail. I shall first discuss the evidence supporting a putative role of prostaglandins in the antihypertensive action of captopril.

To understand the mechanism by which captopril stimulates prostaglandin synthesis it is important to understand the biosynthetic pathway of prostaglandins. This pathway has been worked out in many laboratories and I refer the reader to Samuelsson, Ramwell and Paoletti (1976-1983), for a more detailed review of the following summary.

Arachidonic acid (AA) is a poly-unsaturated fatty acid stored in the phospholipid storage pool of every cell in the body. AA is released from the cell via the activation of a phospholipase (PL) enzyme, predominantly PLA_2. AA is then converted to prostaglandin (PG) G_2 by a cyclo-oxygenase enzyme, which can be acetylated and inactivated by aspirin and indomethacin. PGG_2 can be converted to PGE_2

or $PGF_{2\alpha}$ by peroxidation and isomerization. PGE_2 is a potent vasodilator, while $PGF_{2\alpha}$ is a vasoconstrictor. Both, however, are rapidly metabolised in a single passage through the pulmonary vasculature and their vascular effects are probably not physiologically significant, although locally synthesised PGE_2 and $PGF_{2\alpha}$ may have a significant effect on individual vascular beds. PGG_2 can also be converted to Thromboxane A_2 by a thromboxane synthetase or to prostacyclin (PGI_2) by a prostacyclin synthetase. Thromboxane A_2 is predominantly synthetised in the platelet and is a potent stimulant of platelet aggregation, and although it can be synthesised by other tissues and is a potent vasoconstrictor, it is probably not a vasoconstricting hormone. PGI_2 is produced predominantly by the vascular endothelium and is a potent and probably important vasodilating hormone and inhibitor of platelet aggregation.

Captopril stimulates prostaglandin biosynthesis through an activation of PLA_2 and therefore through an increase in the rate of release of AA (Zusman, 1984; Galler, Folkert and Schlondorff, 1981). If an increase in the vasodilating prostaglandins, PGE_2 and PGI_2 in particular, was the only or most significant effect of captopril on prostaglandin activity, then this would indeed contribute to the antihypertensive activity of captopril. In the kidney, the vascular endothelium, mesangial cells of the glomerulus, epithelial cells of the Bowmans capsule, the renomedullary interstitial cells and the epithelial cells of the collecting tubules have all been shown to be capable of synthesising prostaglandins (Zusman, 1983). Zusman (1984) demonstrated that captopril stimulated PGE_2 biosynthesis thirty-fold in the renomedullary interstitial cell tissue culture, and it has also

been shown to increase PGI_2 and PGE_2 biosynthesis in isolated renal glomeruli, and to stimulate the biosynthesis of these vasodilating prostaglandins in vascular endothelium.

Captopril may also indirectly increase prostaglandin biosynthesis through increasing plasma bradykinin activity. Angiotensin converting enzyme (ACE) is identical to the kinase which inactivates bradykinin and ACE inhibitors therefore impair the catabolism of bradykinin and have been shown to increase plasma kinin activity (McCaa, Hall and McCaa, 1978; Swartz, Williams, Hollenberg, Moore and Dluhy, 1979; Swartz, Williams, Hollenberg, Levine, Dluhy and Moore, 1980). Bradykinin itself is a potent vasodilator and has been shown to increase biosynthesis and release of vasodilating PGs from renal and other tissues and this effect is enhanced by captopril (Murthy, Waldron and Goldberg, 1978). Kinins, however, are local modulators rather than circulating hormones and many other enzymes are capable of inactivating them through kinase activity. It is probably for these reasons that some workers have been unable to demonstrate a significant change in plasma bradykinin levels following captopril administration (see Zusman, 1984). Administration of antikinin antibodies in spontaneously hypertensive and 2-kidney, 1-clip hypertensive rats has been shown to attenuate the response to captopril (Carretero, Scicli and Maitra, 1981), which may indicate that an increase in tissue kinins may contribute to captopril's hypotensive action, or indicate a parallel hypotensive action of kinins themselves.

So captopril is capable of increasing PG biosynthesis both

directly and indirectly. Vinci, Horowitz, Zusman, Pisano, Catt and Keiser (1979) first demonstrated that arterial concentrations of PGE_2 were increased during ACE inhibitor administration. Swartz, Williams, Hollenberg, Crantz, Levine, Moore and Dluhy (1980) reported that captopril increased the excretion of the 13,14 dihydro-15-keto metabolite of PGE_2 in subjects on both low and high sodium diets and that the depressor effect of captopril correlated closely with the changes in the excretion of this metabolite, but not with plasma bradykinin levels. Abe, Ito, Sato, Haruyama, Sato, Omata, Hiwatari, Sakurai, Imai and Yoshinaga (1980) also reported an increase in urinary PGE_2 secretion following captopril administration in essential hypertensive patients, and indomethacin reduced the hypotensive effect of captopril. Silberbauer, Stanek and Templ (1982) reported that after acute indomethacin pretreatment, the acute hypotensive effect of captopril was reduced in both normotensive and essential hypertensive patients. Indomethacin also reduced the changes in AII levels and plasma renin activity observed following captopril administration.

From the evidence discussed here it could be concluded that the ability of captoptil to increase circulating PG synthesis contributes to its hypotensive effect.

I shall now discuss the actions of PGs on -: 1) Sympathetic nerve activity, 2) Adrenal medullary secretion of catecholamines, 3) Renal renin release and the possible interactions of captopril on these actions of PGs.

1. There is much evidence to suggest that PGs exert an

inhibitory effect on the release of noradrenaline from sympathetic nerve terminals. This effect was first demonstrated by Hedquist and Brundin (1969) and Hedquist (1969). They reported that in the isolated spleen of the cat, both PGE_1 and PGE_2 reduced the overflow of noradrenaline evoked by sympathetic nerve impulses. Such impulses stimulate the synthesis of PGE_2 and PGE_1 (Hedquist, 1970; Hedquist, 1973) and so these PGs, mobilised by sympathetic nerve stimulation, were proposed to exert a negative feedback on further release of noradrenaline.

PGE_2 has been shown to inhibit the stimulated release of noradrenaline in the cat mesenteric artery and spleen, rat iris and cerebral cortex, guinea-pig vas deferens, rabbit pulmonary artery, kidney and oviduct and human arteries, veins and oviduct. PG synthesis inhibitors enhance the stimulated release of noradrenaline in these preparations (see Starke, 1977b). Most of the evidence is compatible with the idea that activation of pre-synaptic PG receptors decreases the availability of Ca^{++} for stimulus-secretion coupling (see Starke, 1977b).

So PGE_1 and PGE_2 appear to inhibit the release of noradrenaline from post-ganglionic nerve terminals. If captopril increases the biosynthesis of PGs, it may be expected to enhance the inhibitory effects of PGs on stimulation-evoked noradrenaline release. At the blood vessels, this would result in a reduction of sympathetic vasoconstriction both by reducing circulating AII (which facilitates stimulation-evoked noradrenaline release (see Part 3)), by increasing PG release and also by increasing circulating levels of vasodilating

PGs such as PGE_2 and PGI_2. This is compatible with a contributary role of PGs in the hypotensive action of captopril.

PGs appear to exert a quite different effect on sympathetic ganglia.

2. I found much contradiction in the literature on the effects of PGs on the adrenal medulla.

In 1968, Kayaalp and Turker demonstrated that PGE_1, acetylcholine (Ach) and dimethylphenylpiperazinium (DMPP) increased the perfusion pressure in the dog autoperfused hind limb due to release of catecholamines from the adrenal medulla. Hemicholinium-3 (HC-3), which inhibits the synthesis of Ach abolished the effect of PGE_1 but did not affect the response to Ach or DMPP. This indicated that PGE_1 was exerting a facilitatory, pre-synaptic effect on Ach release from the splanchnic nerve. This facilitatory effect of PGE_1 on cholinergic nerves is supported by the work of Brody and Kadowitz (1974). It is, however, contradictory to the evidence presented by Miele (1969) and Hedquist (1973) who demonstrated that PGE_1, PGE_2 and $PGF_{2\alpha}$ did not alter the adrenal secretion of catecholamines elicited by splanchnic nerve stimulation.

Experiments using isolated adrenal glands also provide contradictory evidence. Work previously carried out in this laboratory (Ellis, 1983), demonstrated that indomethacin had no effect on basal release of catecholamines from the isolated dog adrenal gland, but did reduce the stimulated release in response to nicotine. PGE_2 also

induced a marked increase in catecholamine secretion. Nicotinic stimulation of the gland has been shown to induce release of $PGF_{2\alpha}$ from the adrenal medulla (Ramwell, Shaw, Douglas and Poisner, 1966). This evidence suggests that synthesis of stimulation-induced PGs in the adrenal medulla results in a facilitatory effect of PGs on stimulation-evoked catecholamine release.

Damas (1974) has suggested that indomethacin may exert a direct stimulatory effect on catecholamine release from the rat adrenal medulla not associated with its inhibitory effect on PG synthesis. Feuerstein, Feuerstein and Gutman (1979) reported that PGE_1 and PGE_2 inhibit the release of catecholamines from the rat adrenal medulla in vitro, whereas indomethacin enhanced the release. These results are obviously in direct conflict with those of Ellis (1973). They are supported work previously presented by Gutman and Boonyaviroj who demonstrated that the Ca^{++} dependant outflow of catecholamines from slices of rat adrenal glands was inhibited by PGE_1 and PGE_2 (Gutman and Boonyaviroj, 1975; Boonyaviroj and Gutman, 1975).

In vivo experiments also provide conflicting evidence. Ellis (1973) demonstrated that indomethacin either reduced both the resting release of catecholamines and the reflex release in response to baroreceptor stimulation, or had no effect. This would suggest that PGs facilitate adrenal release of catecholamines in the dog. This suggestion is supported by Feuerstein, Jimerson and Koplin (1981) who demonstrated that PGE_2 infusion increased circulating noradrenaline and adrenaline and improved survival of dogs following haemorrhagic shock. This is contradictory to the evidence they previously

presented, using the isolated gland (see above). PGE_1, PGE_2 and $PGF_{2\alpha}$ have been reported to have no effect on the basal outflow of catecholamines or release induced by various secretagogues, from the cat adrenal medulla (Miele, 1969; Hedquist, 1973).

The in vivo experiments support a facilitatory effect of PGs on adrenomedullary release of catecholamines. I consider that in vivo experimental evidence is more reliable, as in vitro experiments involve many more unphysiological procedures which could effect the results obtained. I consider that the discussed evidence suggests-:

1. Resting adrenal release of catecholamines may not be affected by PGs.
2. Stimulation-induced, neuronally produced PGs may facilitate Ach from the splanchnic nerves, and hence adrenal catecholamine release.
3. Physiological stimuli such as baroreceptor stimulation may induce PG biosynthesis within the adrenal medulla, and this may facilitate adrenal catecholamine release.

3. PGE_2, $PGF_{2\alpha}$ and PGA_2 have all been isolated from the renal medulla (Lee, Crowshaw, Takman, Attrep and Gougoutas, 1967) but PGI_2 is the prominant PG synthesised in the renal cortex and it stimulates renin release from the kidney both in vivo and in vitro (Frolich, 1980). Frolich (1980) also demonstrated that indomethacin decreases PGI_2 secretion and plasma renin activity (PRA) from the kidney. Arachidonic acid has been shown to be a strong stimulus for renin release in the rabbit (Larsson and Anggard, 1974) but Frolich suggests that the actions of PGs on renin release are species specific. For

example, where β-receptor mediated release is concerned, in man, release is independant of PG effects, in the rat, PGs facilitate renin secretion (Campbell, Graham and Jackson, 1979) and in the dog, although the β-receptor mediated release is independant of PGs (Berl, Henrich, Erickson and Schrier, 1979), baroreceptor stimulated release requires a PG step (Estilo and Cottrell, 1982).

PGs are important local modulators of the effects of pressor hormones (McGiff, Crowshaw, Terragno and Lonigro, 1970) and it has been demonstrated in sodium depleted rats, that indomethacin may enhance the pressor responsiveness of AII in situations where plasma AII levels are elevated (Smyth and Fung, 1984). Captopril can prevent this effect of indomethacin. This suggests that PGs inhibit the pressor activity of AII. This could be explained by PGs and AII exerting opposing inhibitory and facilitatory, respectively, effects on noradrenaline release from sympathetic nerves (previously discussed).

From the evidence discussed, by increasing PG release, captopril could have the following effects-:

1. By increasing the PG negative feedback on sympathetic nerves - reduce sympathetically induced vasoconstriction.

2. By increasing vasodilating PGs - reduce vasoconstriction.

3. By increasing stimulation-induced adrenal PG release - increase PG facilitation of adrenal catecholamine release in

situations of cardiovascular stress eg. haemorrhage.

4. By increasing PGs inhibitory effects on AII pressor activity, and by inhibiting AII synthesis - reduce vasoconstriction.

Effects 1,2 and 4 would all contribute to the hypotensive effect of captopril, but 3 is not compatible with the inhibition, by captopril, of reflex adrenal catecholamine release that I have demonstrated during this research project.

The effect of captopril on PG release is of clinical importance in patients receiving captopril ie. indomethacin has been shown to decrease the hypotensive effect of captopril (Silberbauer etal, 1982). It is also important to consider the effect of co-administration of captopril and PG synthesis inhibitors on the reflex release of catecholamines induced by cardiovascular stresses such as haemorrhage, hypoglycaemia, exertion etc. Captopril and, apparently, indomethacin both inhibit such reflex releases of catecholamines, and so patients receiving both may lack this vital physiological compensatory mechanism.

The aims of this series of experiments were to answer the following questions-:

1. Does prior administration of indomethacin increase the inhibitory effects of captopril on adrenal catecholamine release, before and after baroreceptor stimulation, in the anaesthetised dog ?

2. Does prior administration of indomethacin increase the inhibitory effects of captopril on the catecholamine release evoked by splanchnic nerve stimulation, from the denervated adrenal gland ?

3. Does prior administration of indomethacin reduce the hypotensive action of captopril ?

Part 5 - Results

1. The effect of indomethacin, followed by captopril administration on adrenal catecholamine release before and after baroreceptor stimulation.

The effect of indomethacin ($5mgkg^{-1}$), followed by captopril administration, on adrenal catecholamine release before and after six minute baroreceptor stimulation, and the change in catecholamine release induced by baroreceptor stimulation, was studied in dogs 41-44. The results are shown in table 5.1 and illustrated in figure 5.1.

The results show that indomethacin reduced the resting release of catecholamines, and significantly inhibited the reflex release induced by baroreceptor stimulation. Captopril had little additional effect on resting release, but further reduced the reflex release, which was effectively abolished 5-6 minutes after the onset of baroreceptor stimulation.

These results are compatible with the hypothesis that prostaglandins exert a facilitatory effect on both resting and reflex adrenal catecholamine release. Indomethacin may potentiate the effect of captopril on adrenal catecholamine release.

Table 5.1

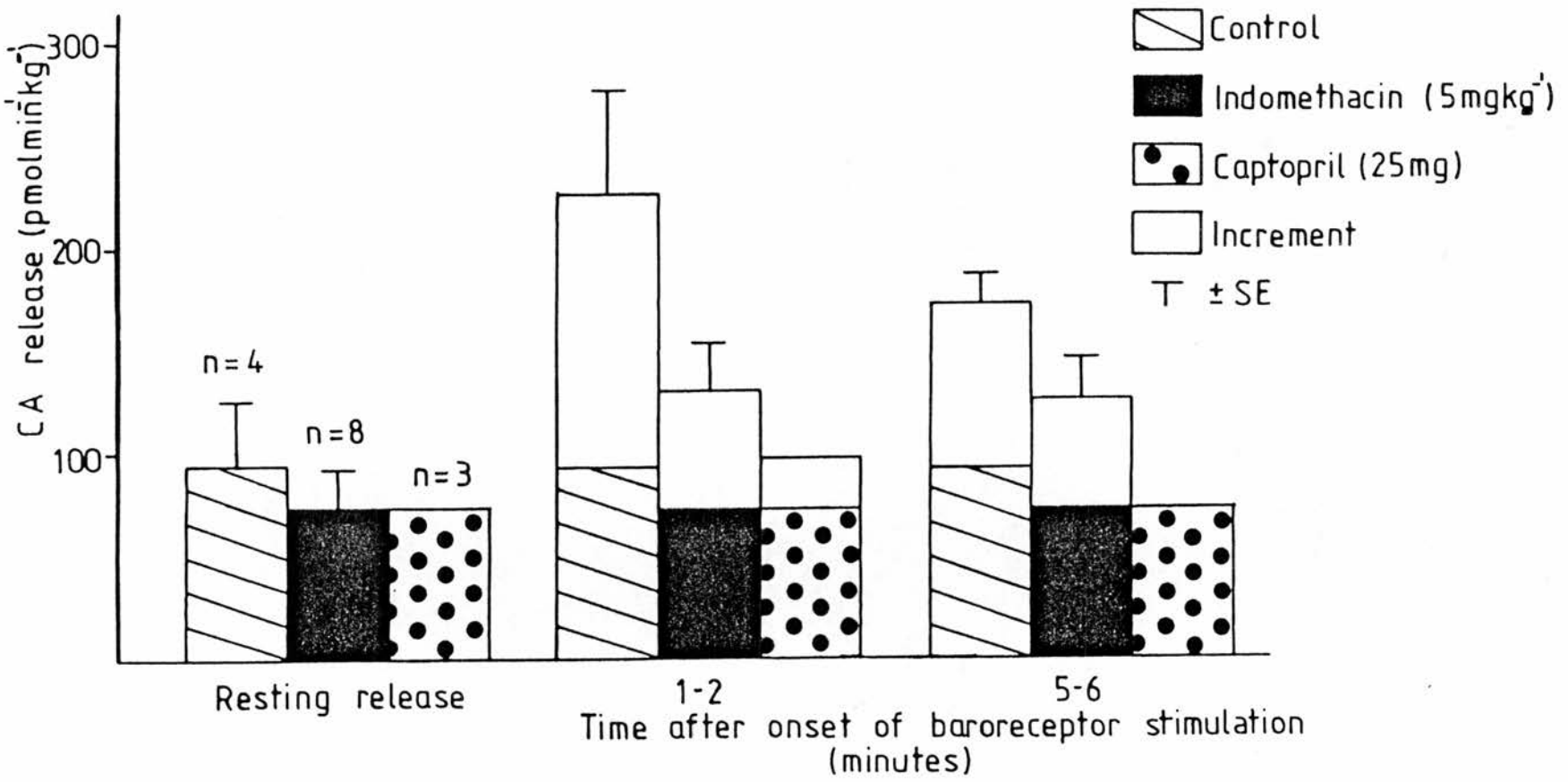
The effect of indomethacin, followed by captopril administration, on catecholamines output before and after baroreceptor stimulation (BRS)

Drug Treatment	Time after onset of BRS (mins)	Total catecholamine output ($pmolmin^{-1}kg^{-1}$) Dog41	Dog42	Dog43	Dog44	Mean ± SE
Control	0	65	117	162	31	93.8 ± 28.8
	1-2	115	387	320	90	228.0 ± 73.9
	5-6	157	198	202	138	173.8 ± 15.7
Indomethacin ($5mgkg^{-1}$)	0	45,55	213,107	60,59	10,40	73.6 ± 22.1
	1-2	37,80	291,288	60,118	70,99	130.4 ± 35.8*
	5-6	27,105	251,279	79,137	71,76	128.1 ± 31.9*
Captopril (25mg)	0	15	152	52		73.0
	1-2	17	191	87		98.3
	5-6	27	128	66		73.7
		Change in catecholamine output during BRS				
		Dog41	Dog42	Dog43	Dog44	Mean ± SE
Control	0					
	1-2	50	270	158	59	134.3 ± 51.0
	5-6	92	81	40	107	80.0 ± 14.4
Indomethacin ($5mgkg^{-1}$)	0					
	1-2	8,25	78,181	0,59	60,59	56.8 ± 20.8**
	5-6	18,50	38,172	19,78	61,36	54.5 ± 19.6
Captopril (25mg)	0					
	1-2	2	39	35		25.3
	5-6	12	-24	14		0.7

Statistical significance (indomethacin data compared with control data)-:
* - $p > 0.05$
** - $p > 0.02$

Figure 5·1

The effect of indomethacin, followed by captopril administration on adrenal catecholamine (CA) release, before and after baroreceptor stimulation.



2. The effect of indomethacin, followed by captopril administration, on adrenal catecholamine release before and after splanchnic nerve stimulation.

The effect of indomethacin, followed by captopril administration, on adrenal catecholamine release before and after splanchnic nerve stimulation, from the denervated adrenal gland, was studied in dogs 45-47. The results are shown in table 5.2 and illustrated in figure 5.2.

The results show that indomethacin increased resting catecholamine release in dogs 45-47, but reduced the release evoked by splanchnic nerve stimulation. Captopril reduced the resting release of catecholamines and further reduced the release evoked by splanchnic nerve stimulation.

Comment

It is difficult to explain why indomethacin increases the resting release in these dogs, while decreasing resting release in dogs 41-44. It is possible that as the adrenal glands in dogs 41-44 were innervated, that indomethacin inhibited a neural component of resting catecholamine release. As discussed in the introduction, Ellis (1983) also reported that indomethacin had varying effects on resting catecholamine release, while usually inhibiting reflex release. These results show that indomethacin did reduce the release of catecholamines evoked by splanchnic nerve stimulation, indicating that prostaglandins have a facilitatory effect on this release.

Table 5.2

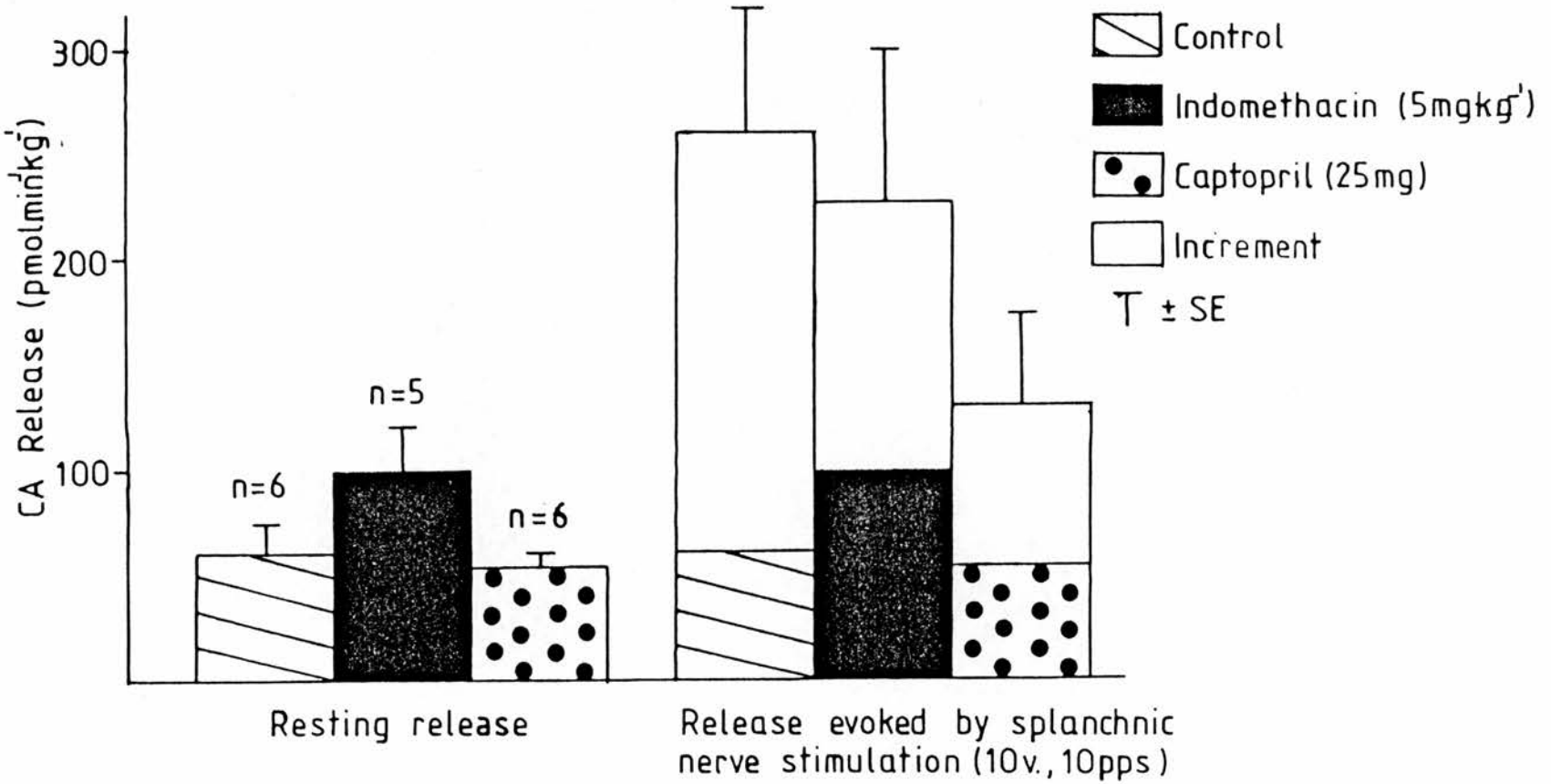
The effect of indomethacin, followed by captopril administration, on catecholamines output before and after splanchnic nerve stimulation (SNS)

Drug Treatment	Function	Total catecholamine output ($pmolmin^{-1}kg^{-1}$) Dog45	Dog46	Dog47	Mean ± SE
Control	C	46,29	8,92	85,94	59.0 ± 14.9
	+S	63,56	356,423	296,361	259.2 ± 65.3
Indomethacin ($5mgkg^{-1}$)	C	69,80	52	135,159	99.0 ± 20.5
	+S	47,50	382	387,266	226.8 ± 76.0
Captopril (25mg)	C	48,87	40,32	55,51	52.2 ± 7.7
	+S	53,74	272,240	81,62	130.3 ± 40.2
		Change in catecholamine output during SNS			
		Dog45	Dog46	Dog47	Mean ± SE
Control	+S	17,27	348,331	211,267	200.2 ± 59.8
Indomethacin ($5mgkg^{-1}$)	+S	-22,-30	332	252,107	127.8 ± 72.4
Captopril (25mg)	+S	5,-13	232,208	26,11	78.2 ± 45.3

C = Control +S = During splanchnic nerve stimulation

Figure 5·2

The effect of indomethacin, followed by captopril administration on adrenal catecholamine (CA) release, before and after splanchnic nerve stimulation.



3. The effect of indomethacin, followed by captopril administration, on systemic blood pressure.

Silberbauer etal (1982) have demonstrated that indomethacin can blunt the acute hypotensive effect of captopril in both normotensive and hypertensive patients. It was of interest, therefore to study the effect of indomethacin on the resting systemic blood pressure (SBP) in the anaesthetised dog. The effect of indomethacin, followed by captopril administration, on resting SBP was studied in dogs 41-47. The results are shown in table 5.3.

The results show that indomethacin had no significant effect on resting SBP. It can be calculated from the results that captopril induced a fall in resting SBP of 11.1 ± 3.6mmHg (n=14) when administered after indomethacin. It can be calculated from table 1.10 and table 3.6 that captopril induced a fall in resting SBP of 15.3 ± 3.0mmHg (n=17) and 13.1 ± 2.9 (n=13) respectively, when administered before any other drug. This indicates that indomethacin does not significantly blunt the hypotensive effect of captopril in the anaesthetised dog.

4. The effect of indomethacin, followed by captopril administration, on adrenal blood flow.

Work previously carried out in this laboratory (Ellis, 1983) indicated that prostaglandins may play a role in maintaining adrenal blood flow in the anaesthetised dog.

The effect of indomethacin, followed by captopril administration,

Table 5.3

The effect of indomethacin, followed by captopril administration on resting systemic blood pressure, adrenal blood flow and haematocrit in dogs 41-47 (D41-47)

Control

	D41	D42	D43	D44	D45	D46	D47	Mean ± SE
SBP	130	120	155	80	125,115	85,85	85,85	106.5 ± 8.2 (n=10)
BF	6.1	4.2	5.0	3.0	1.7,0.9	2.4,2.2	1.8,1.3	2.9 ± 0.5 (n=10)
HAEM	57	55	54	50	53,56	50,55		53.8 ± 0.9 (n=8)
VISC	6.2	5.8	5.8	5.0	5.7,6.0	5.0,6.0		5.7 ± 0.2 (n=0.2)
BFxVISC	38	24	29	15	9.7,5.4	12,13		

Indomethacin

SBP	135,135	85,70	140,120	70,70	120,125	90,70	75,85	99.3 ± 7.5 (n=14)
BF	3.6,3.8	1.9,1.0	3.2,2.9	1.5,1.2	1.7,1.5	1.8,0.9	0.9,0.8	** 1.9 ± 0.3 (n=14)
HAEM	52,50	47,40	47,52	40,42	47,53	44,56		**47.5 ± 1.5 (n=12)
VISC	5.2,5.0	5.0,4.2	5.0,5.2	4.0,4.4	5.0,5.7	4.5,6.0		4.9 ± 0.2 (n=12)
BFxVIS	19,19	9.5,4.2	16,15	6,5.2	8.5,8.6	8.1,5.6		

Captopril

SBP	115	65	105	70	105,110	65,65	90,90	*88.0 ± 6.4 (n=10)
BF	3.5	3.5	4.6	3.9	2.5,2.1	1.8,1.9	1.1,0.7	* 2.6 ± 0.4 (n=10)
HAEM	40	34	43	38	40,38	33,26		**36.5 ± 1.9 (n=8)
VISC	4.0	3.6	4.4	3.8	4.0,3.8	3.5,3.0		3.8 ± 0.1 (n=8)
BFxVISC	14	13	20	15	10,8	6.3,5.7		

SBP = Systemic blood pressure
BF = Adrenal blood flow ($mlmin^{-1}$)
HAEM = Haematocrit (%)
VISC = Relative viscosity
Statistical significance (Captopril SBP data vs Indomethacin SBP data
Indomethacin BF data vs Control BF data
Indomethacin HAEM data vs Control HAEM data
Captopril BF data vs Indomethacin BF data
Captopril HAEM data vs Indomethacin HAEM data)-:

* = $p > 0.01$
** = $p > 0.001$

on adrenal blood flow was analysed in dogs 41-47. The results are shown in table 5.3.

The results show that indomethacin reduced adrenal blood flow in these dogs. After captopril administration, the blood flow increased.

Comments

These results suggest that prostaglandins may play a role in maintaining adrenal blood flow. As has been shown in Part 1, the adrenal blood flow changes seen after captopril may be partly due to dilution of the blood by dextran, resulting in a reduction of haematocrit (table 1.13). Table 5.3 also shows the effect of indomethacin and captopril on adrenal venous blood haematocrit. Indomethacin alone can be seen to cause a reduction in haematocrit and no dextran was infused at this time in the experiments. This infers that the facilitatory effect of prostaglandins on adrenal blood flow is not related to a reduction in haematocrit (see discussion). Table 5.3 also shows that in these dogs, after indomethacin administration, captopril induced a further fall in haematocrit, which may be due to dilution of the blood by dextran.

5. The effect of indomethacin, followed by captopril administration, on adrenal vascular resistance.

The results have shown that captopril increases adrenal blood flow and indomethacin decreases adrenal blood flow. It was of interest to investigate whether this effect could be explained by

changes in vascular resistance ie: does captopril vasodilate and lower resistance or indomethacin vasoconstrict and increase resistance ?

This was investigated by constructing a linear regression graph for systemic blood pressure vs adrenal blood flow x relative viscosity. The reasoning behind this is as follows-:

Vasodilation induces a higher blood flow for a given pressure and lowers vascular resistance. Vasoconstriction has opposing effects. This is expressed by Poiseuilles law for a steady flow-:

1. $R = P/F$

2. $$F = \frac{\Delta P \times Ra^4}{L \times V}$$

3. $$R = \frac{\Delta P}{F} = \frac{L \times V}{Ra^4}$$

R = resistance, F = flow, P = pressure, ΔP = pressure gradient, Ra = radius of vessel, L = length of vessel, V = viscosity of blood.

So a change in resistance could be due to a change in the geometry of the vessel or to a change in the viscosity of the blood. The length of the vessel will not change, while viscosity and the radius may. To investigate if a change in resistance is due to a change in the radius of the vessel, it is necessary to correct for possible changes in the viscosity, ie-: (over)

4. $R = \frac{\Delta P}{F \times V} = \frac{L}{Ra^4}$

A graph of systemic blood pressure vs flow, corrected for changes in viscosity will indicate changes in resistance which are due to the geometric component. This enables a comparison of the effects of captopril and indomethacin in terms of vasodilation and vasoconstriction.

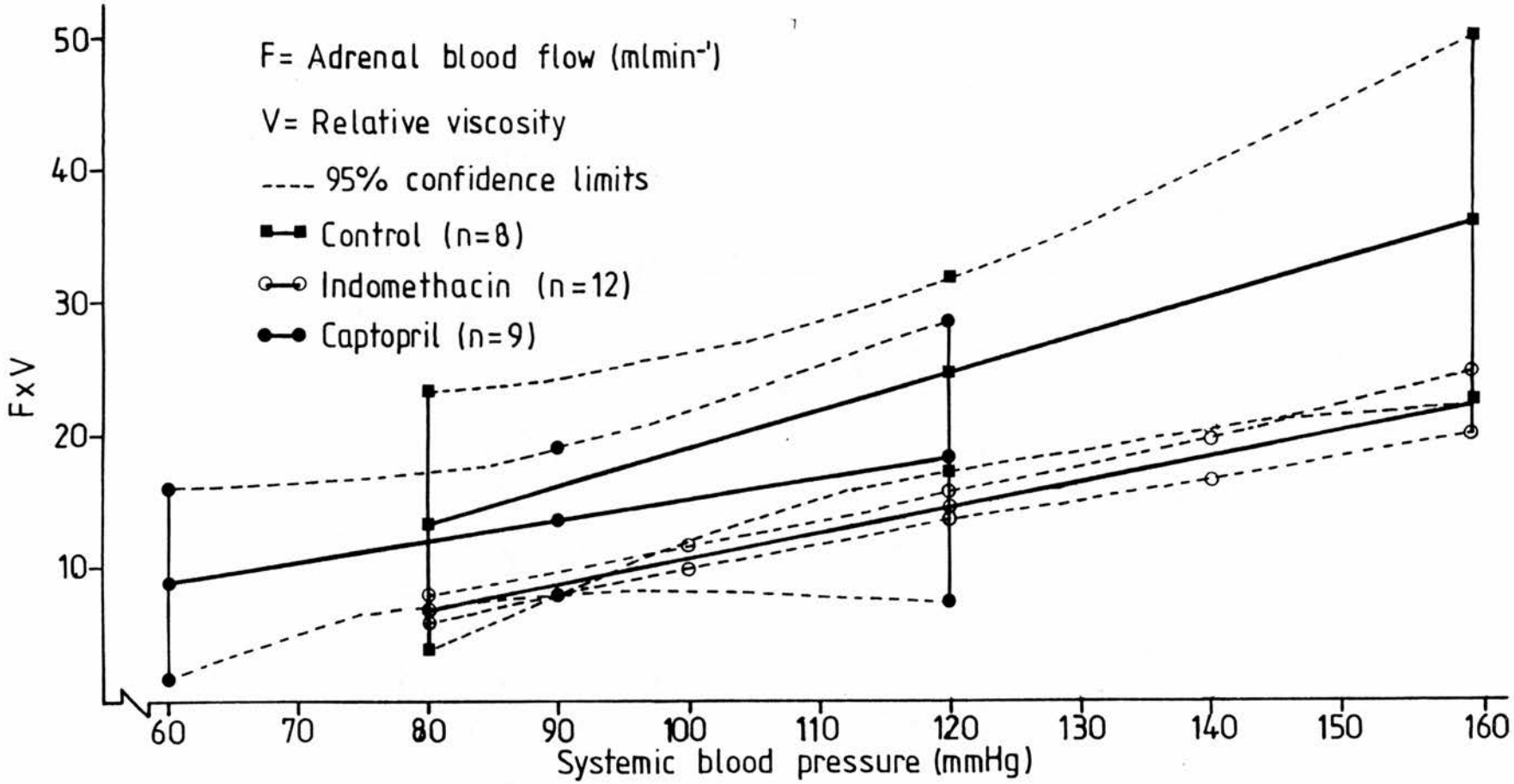
The data used to plot this graph is shown in table 5.3. Viscosity was determined from a graph in "The mechanics of the circulation" by Caro, Pedley, Schroter and Seed, 1978, page 179. This graph plots haematocrit (%) vs relative viscosity in human blood. The viscosity of the adrenal blood was determined from the calculated haematocrit shown in table 5.3. It is emphasised that the relative viscosity values are therefore approximated values. The linear regression graph with 95% confidence limits is shown in figure 5.3.

Comment

Figure 5.3 shows that the 95% confidence limits are large for the control and post captopril data, but does indicate that a degree of autoregulation exists in the adrenal gland, as has been reported in the perfused kidney (Dighe, Hall, Smith and Ungar, 1977), ie, flow is maintained despite reductions in systemic blood pressure. The graph shows that indomethacin shifts the line to the right, significantly between 100 and 145mmHg. Subsequent captopril administration shifted the regression line back towards the left, relative to indomethacin.

Figure 5·3

Linear regression lines for systemic blood pressure vs. adrenal blood flow x relative viscosity before and after indomethacin, and subsequent captopril, administration.



These results suggest that indomethacin induces a vasoconstriction. Indomethacin induced a significant reduction in haematocrit, but the results show that the vasoconstriction induced by indomethacin overcame this effect, which reinforces the evidence suggesting indomethacin induces a vasoconstriction.

Captopril, administered after indomethacin, partially countered the effect of indomethacin, but there is no evidence for a significant vasodilation by captopril. These observations may explain why indomethacin reduced adrenal blood flow and why flow was increased following subsequent captopril administration. The results suggests that indomethacin exerts a vasoconstriction which would contribute to the reduction in adrenal blood flow observed following indomethacin administration. The effect of captopril on adrenal blood flow was probably due to the reduction in haematocrit observed, although the possibility that captopril exerts a vasodilation cannot be definately ruled out. As has been mentioned, there is evidence that prostaglandins may play a role in maintaining adrenal blood flow. These results suggest that they may exert a tonic vasodilation on adrenal blood vessels which would contribute to this role.

6. The effect of indomethacin, followed by captopril administration, on plasma renin activity.

The effect of indomethacin, followed by captopril administration, on plasma renin activity (PRA) was studied in dogs 41-45. The results are shown in table 5.4.

Table 5.4

The effect of indomethacin, followed by captopril administration on plasma renin activity in dogs 41-45 (D41-45)

Drug Treatment	Plasma renin activity ($ngAIml hr^{-1}$) D41	D42	D43	D44	D45	Mean ± SE
Control	28.2	7.6	21.5	13.4	8.5	15.8 ± 4.0
Indomethacin ($5mgkg^{-1}$)	14.8, 7.6	17.5, 28.2	25.1, 24.2	15.7, 11.2	6.7, 12.5	16.4 ± 2.3
Captopril (25mg)	14.3	28.2	38.1	14.3	19.3	22.8 ± 4.6*

Statistical significance (Captopril data vs indomethacin data)-:
* = $p > 0.01$

The results show that indomethacin had no effect on PRA and the subsequent captopril administration induced the increase in PRA normally detected after captopril administration.

7. The effect of flurbiprofen on adrenal catecholamine release, before and after splanchnic nerve stimulation

In one dog, dog 52, the effect of an alternative cyclo-oxygenase inhibitor, flurbiprofen, on adrenal catecholamine release was analysed. The results are shown in table 5.5.

The results show that flurbiprofen reduced the resting output of catecholamines and the release evoked by splanchnic nerve stimulation. These were further reduced by captopril.

These results are similar to those obtained for indomethacin and confirm that the effects of indomethacin were due to its ability to inhibit cyclo-oxygenase and not to some other effect.

Table 5.5

The effect of flurbiprofen on adrenal catecholamine output, before and after splanchnic nerve stimulation in dog 52

Drug Treatment	Function	CA output ($pmolmin^{-1}kg^{-1}$)	CA Increment from control	Mean CA output	Mean Increment
Control	C	482,158		320	
	+S	575,453	93,295	514	194
Flurbiprofen ($5mgkg^{-1}$)	C	72,30		51	
	+S	253,78	181,48	156	105
Captopril (25mg)	C	55,43		49	
		68,88	13,45	78	29

C = Control
+S = During splanchnic nerve stimulation (10v,10pps)
CA = Catecholamine

Part 5 - Summary of results

1. In four anaesthetised dogs, indomethacin (5mgkg^{-1}) reduced the resting adrenal output of catecholamines from the innervated adrenal gland and reduced the reflex release of catecholamine induced by baroreceptor stimulation. Subsequent administration of captopril (25mg) further reduced the reflex release.

2. In three more anaesthetised dogs, indomethacin increased resting adrenal catecholamine output from the denervated adrenal gland and reduced the release evoked by splanchnic nerve stimulation. Subsequent administration of captopril reduced the resting release and further reduced the release evoked by splanchnic nerve stimulation.

3. Indomethacin did not affect resting systemic blood pressure and did not blunt the hypotensive action of captopril.

4. Indomethacin reduced adrenal blood flow and this was increased following captopril administration.

5. Indomethacin induced an increase in vascular resistance and this vasoconstriction probably accounts for the decrease in adrenal blood flow induced by indomethacin.

6. Indomethacin did not alter plasma renin activity, and did not affect the increase in plasma renin activity which occurs after captopril administration.

7. In one dog, flurbiprofen (5mgkg^{-1}) had a similar effect to indomethacin on adrenal catecholamine release.

Part 5 - Discussion of results

The results show that indomethacin either reduced (innervated glands) or increased (denervated glands) resting adrenal catecholamine release and reduced the reflex release of catecholamines induced by baroreceptor stimulation and the release evoked by splanchnic nerve stimulation from denervated glands.

Ellis (1983) also observed varying effects of indomethacin on resting release of catecholamines. He reported that indomethacin had no effect on the resting release of catecholamines from the isolated dog adrenal gland but did reduce the stimulated release in response to nicotine. He also reported that, in the anaesthetised dog, indomethacin either reduced or had no effect on the resting release.

The results suggest that prostaglandins may facilitate adrenal release of catecholamines in response to baroreceptor stimulation. They also suggest that prostaglandins may facilitate splanchnic nerve activity or splanchnic nerve stimulation-induced adrenal catecholamine release. The results therefore support the evidence discussed in the "Introduction and literature review" which were "for" a facilitatory role of prostaglandins in the reflex release of catecholamines from the adrenal medulla in response to cardiovascular stress.

The results of the splanchnic nerve stimulation studies are compatible with the conclusions of Kayaalp and Turker (1968) and Brody and Kadowitz (1974) (see "Introduction and literature review", that prostaglandins (particularly PGE_2) may exert a facilitatory,

presynaptic effect on stimulated acetylcholine release from the splanchnic nerve. This would explain the reduction in splanchnic nerve-stimulated adrenal catecholamine release from the denervated adrenal gland, following indomethacin, observed in my experiments. The results suggest that coadministration of prostaglandin synthesis inhibitors may potentiate the inhibitory effect of captopril on reflex adrenal catecholamine release in response to cardiovascular stress. This could be of clinical importance. It should be considered that, in patients receiving such a combination, this important physiological response to cardiovascular stress may be severely impaired.

So the results suggest that, in the anaesthetised dog, prostaglandins exert a facilitatory effect on the reflex and stimulated release of catecholamines in the adrenal gland. Specific binding sites for PGE_2 and PGE_1 have been demonstrated in bovine, ovine and human medullae (Dazord, Morera, Bertrand and Saez, 1974; Karaplis and Powell, 1981).

Indomethacin did not affect the resting systemic blood pressure and had no blunting effect on the hypotensive action of captopril. This indicates that any increase in vasodilating prostaglandin synthesis did not contribute to its' hypotensive action. Silberbauer etal (1982) demonstrated that, in healthy volunteers and patients with essential hypertension, indomethacin significantly blunted the hypotensive effect of captopril. This indicates that, in man, part of the hypotensive effect of captopril may be related to its ability to increase vasodilating prostaglandin secretion. This effect may

therefore be species related. These results could also indicate, however, that prostaglandins and AII have parallel but opposite effects on blood pressure, and the effect of indomethacin may be related to this and not to an effect of captopril on prostaglandin synthesis.

The effects of flurbiprofen were comparable with those of indomethacin which indicates that the effects of indomethacin were related to cyclo-oxygenase inhibition and not to any other effect. Indomethacin has calcium antagonist properties (Northover, 1977) and I particularly wished to omit the possibility that the effect of indomethacin on adrenal catecholamine release was due to calcium antagonism. I could find no reference to flurbiprofen being a calcium antagonist.

The presence of prostaglandins in the adrenal vein may be important, as they may play a role in counteracting the vasoconstriction associated with catecholamine release, which could be damaging to the veins as the concentration of catecholamines is high in the adrenal veins. PGI_2 is a potent inhibitor of platelet aggregation which may occur in the presence of concentrated catecholamines. Prostaglandins may therefore have a protective effect in the adrenal veins. Prostaglandins may play a role in maintaining adrenal blood flow (Ellis, 1983) (see below) and this would contribute to this putative protective role.

The results show that indomethacin reduced adrenal blood flow, and this was restored following captopril administration. This effect

of indomethacin was observed despite the reduction in haematocrit observed following indomethacin administration. Following captopril administration the haematocrit was reduced further. As has been discussed previously, this effect on blood flow after captopril administration could be partly due to dilution of the blood by the infusion of dextran. It was possible, however, that captopril may cause a vasodilation of the adrenal vascular bed which would contribute to its effect on blood flow. AII is a potent vasoconstrictor, and it is possible that a non-pressor level of circulating AII may exert a degree of tonic vasoconstriction, or facilitate such a tonic vasoconstriction by the sympathetic nervous system (see Part 3). Captopril may abolish such an effect and lower vascular resistance. This would contribute to its effect on adrenal blood flow.

The results suggest, however, that the reduction in adrenal blood flow observed after indomethacin administration was due to vasoconstriction, but there was no evidence for a significant effect of captopril on vascular resistance. This suggests that much of the increase in blood flow observed following captopril administration was due to the reduction in haematocrit, probably due to dilution of the blood by dextran. It is possible that prostaglandins may exert a degree of vasodilation, which may be related to their inhibitory effect on noradrenaline release from sympathetic nerve endings (see "Introduction and literature review").

PGI_2 has been shown to reduce adrenal vascular resistance in maternal and foetal sheep (Phernetton and Rankin, 1979). Houck and

Lutherer (1981) and Ellis (1983) have also reported a reduction of canine adrenal blood flow with indomethacin. In addition, in the dog, PGs E_1, E_2, A_1 and A_2 administered by close arterial infusions that do not affect systemic blood pressure, increase blood flow in carotid, renal, femoral, brachial and coronary arteries (Nakano, 1968). Prostaglandins A_1, A_2, B_1, E_2 and $F_{2\alpha}$ also increase forearm blood flow in man (Collier, Karim, Robinson and Somers, 1972) and PGE_1 increases lower limb blood flow in man (Carlson, Ekelund and Oro, 1969). Prostaglandins therefore increase blood flow in many vascular beds.

My results also indicate that a degree of autoregulation exists in the adrenal gland, the gland maintaining blood flow despite changes in systemic blood pressure. This observation is supported by the work of Houck and Lutherer (1981) who demonstrated that, in the dog, the adrenal gland was able to maintain its blood flow during haemorrhagic hypotension to 50mmHg even in the presence of indomethacin.

The results show that indomethacin did not alter plasma renin activity, or prevent the increase in plasma renin activity induced by captopril. As was discussed in the "Introduction and literature review", prostaglandins have been shown to stimulate renin release, and this effect is probably species specific (Frolich, 1980). The results suggest that in the anaesthetised dog, prostaglandins are not involved in basal renin secretion or in the control of renin release by AII. In the discussion in Part 1, I suggested that part of the increase in plasma renin activity observed after captopril may have been due to its ability to increase prostaglandin synthesis. This may

be true for other species but there was no evidence that this was true in these dogs.

It is of interest that PGE_2 has been shown to stimulate corticosteroid synthesis (Flack, Jessup and Ramwell, 1969; Saruta and Kaplan, 1972; Louis, Challis, Robinson and Thorburn, 1976), and that PGE_2 may be involved in ACTH induced corticosteroid secretion. As my results in "Part 2" have shown, corticosteroids may facilitate catecholamine release and this may be involved in the ability of PGE_2 to increase adrenal catecholamine release. Ellis (1973), however, demonstrated that when PGE_2 was retrogradely infused into the canine adrenal gland, therefore bypassing the adrenal cortex, it still induced a marked increase in catecholamine release. This would suggest that the effect of PGE_2 is not dependant on any effect on corticosteroid secretion, but this remains a point of interest.

In conclusion, in the anaesthetised dog, prostaglandins may facilitate the reflex and stimulated release of catecholamines from the adrenal medulla. There is no evidence that captopril's ability to increase vasodilating prostaglandin secretion contributes to its hypotensive effect in the anaesthetised dog. Prostaglandins may play a role in maintaining adrenal blood flow. Indomethacin reduces adrenal blood flow, and this is probably related to a vasoconstriction. Prostaglandins may exert a tonic vasodilation in the adrenal vascular bed. Prostaglandins do not appear to play a major role in renin release in the anaesthetised dog.

Summary of conclusions

The results suggest that the integrity of the renin-AII system is required for the adrenal gland to respond to baroreceptor stimulation in the anaesthetised dog and cat. A minimum, non-pressor level of AII may be required for the gland to respond to the reflex stimulus, and activation of the renin-AII system is not required for the immediate adrenal response. The effect of AII is likely to be a facilitatory one on adrenal catecholamine release at the level of the adrenal gland and not through central activation of sympathetic drive, although this possibility cannot be ruled out.

In addition to a direct effect on adrenomedullary catecholamine release, AII may exert an indirect effect through stimulation of adrenocorticosteroids, which may then facilitate adrenal catecholamine release. In situations of cardiovascular stress, both the renin-AII system and the pituitary adrenocortical axis may cooperate to increase adrenal catecholamine release until homeostasis is restored.

The facilitatory effect of AII on adrenal catecholamine release does not depend on central activation of sympathetic drive. As with sympathetic nerves and other sympathetic ganglia, AII may facilitate splanchnic nerve activity.

Naloxone increases resting adrenal catecholamine release and the release evoked by both baroreceptor stimulation and splanchnic nerve stimulation, from both innervated and denervated glands, respectively. These effects are opposite to those of captopril. Endogenous opioid

peptides, stored in the chromaffin cells of the adrenal medulla and in splanchnic nerve terminals and released with catecholamines, may exert a tonic inhibitory effect on resting adrenal catecholamine release. Endogenous opiates, released from the adrenal medulla with catecholamines following baroreceptor stimulation or splanchnic nerve stimulation may exert a negative feedback on catecholamine release. The catecholamines stored in splanchnic nerve terminals and released with noradrenaline, may decrease nicotinic stimulation of the adrenal medulla. AII and endogenous opioid peptides therefore exert opposing effects on adrenal catecholamine release and this may provide a "fine tuning" of adrenal catecholamine release.

Captopril may increase adrenal and splanchnic nerve endogenous opioid peptide levels which may contribute to its inhibitory effect on adrenal catecholamine release, but this remains purely speculative.

Captopril exerts a considerable inhibitory effect on both adrenal catecholamine release and systemic blood pressure in the low PRA state. This shows that an elevated AII level is not a requirement for the effectiveness of captopril. This is consistent with the hypothesis that it is a minimum circulating level of AII which is important for the response of the adrenal gland to reflex stimuli and splanchnic nerve activity.

Prostaglandins may facilitate the reflex and stimulated release of catecholamines from the adrenal medulla. There is no evidence that captoprils ability to increase vasodilating prostaglandin secretion contributes to its hypotensive effect in the anaesthetised dog.

Prostaglandins may play a role in maintaining adrenal blood flow. Indomethacin reduces adrenal blood flow, and this is probably related to a vasoconstriction. Prostaglandins may exert a tonic vasodilation in the adrenal vascular bed. Prostaglandins do not appear to play a major role in renin release in the anaesthetised dog.

Appendix 1

Individual dog and cat data tables

Key

(All dogs were foxhounds)

Sex - M = Male

- F = Female

S = Sample number

Drug = Drug administered (i.v.), listed in order of administration.

T

+BRS = Time after onset of baroreceptor administration (minutes)

CPP = Carotid perfusion pressure (mmHg)

SBP = Systemic blood pressure (mmHg)

CA = Adrenal catecholamine output ($pmolmin^{-1}kg^{-1}$)

A = Adrenaline

NA/Na = Noradrenaline

BF = Adrenal blood flow ($mlmin^{-1}$)

PRA = Plasma renin activity in arterial blood ($ngAIml^{-1}hr^{-1}$)

AII = Angiotensin II

pCO_2,

pO_2 = Partial gas pressures (mmHg)

ACTH = Adrenocorticotrophic hormone

(+xmin) = Time after ACTH administration

PPS = Pulses per second

C = Control sample prior to splanchnic nerve stimulation

+ S = During splanchnic nerve stimulation (10 volts, 2 ms and 10 PPS unless stated otherwise

Cortisol conc. = Concentration of cortisol (μgml^{-1}plasma)

Cortico.

conc. = Concentration of corticosterone ($\mu g ml^{-1}$ plasma)

(All catecholamine samples lost) refers to samples lost due to breakdown in the assay system.

DOG 1. 26kg, M

DRUG S	T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
Control							
1	0	130	85	20	8.7	4.1	31
2	1-2	90	115	44	3.3	5.0	18
3	5-6	90	115	52	4.0	4.7	30
4	9-10	90	115	62	6.5	4.9	34
5	0	130	85	28	6.0	4.1	
6	1-2	90	105	38	5.5	4.4	
7	5-6	90	105	42	4.0	4.8	
8	9-10	90	105	62	4.5	4.6	
Captopril (25mg)							
9	0	130	55	14	4.5	5.8	64
10	1-2	90	65	16	4.5	6.2	76
11	5-6	90	65	18	8.0	6.9	74
12	9-10	90	65	22	4.5	5.5	86
13	0	130	65	0		6.2	30
14	1-2	90	80	0		6.5	34
15	5-6	90	80	0		6.0	37
16	9-10	90	80	0		5.7	30
AII ($5ngmin^{-1}$)							
17	0	130	65	14	2.5	4.1	
18	1-2	90	80	26	6.0	4.8	
19	5-6	90	80	20	6.0	4.7	
20	9-10	90	80	28	4.6	4.7	

Blood pH and blood gas measurements (n=6) -:

pH = 7.43 ± 0.03

pCO_2 = 27.3 ± 1.70

pO_2 = 100.0 ± 9.4

DOG 2 15kg, F

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
Control							
1	0	130	60	16	3.4	0.8	48
2	1-2	90	120	84	6.6	2.8	35
3	5-6	90	120	54	3.6	1.6	58
4	9-10	90	100	70	4.6	1.1	54
5	0	130	50	22	9.5	0.5	54
6	1-2	90	80	100	5.4	1.7	16
7	5-6	90	80	72	3.6	0.8	54
8	9-10	90	90	130	9.5	2.0	38
Captopril (25mg)							
9	0	130	50	10	6.0	1.9	144
10	1-2	90	80	24	3.3	2.2	51
11	5-6	90	80	38	4.2	1.9	42
12	9-10	90	80	30	7.2	1.5	16
13	0	130	50	10	5.0	1.0	
14	1-2	90	80	24	3.4	1.5	
15	5-6	90	40	8	4.5	0.6	
16	9-10	90	40	16	4.3	0.7	
AII ($5ngmin^{-1}$)							
17	0	130	45	18	4.3	1.9	
18	1-2	90	70	40	3.2	1.8	
19	5-6	90	50	36	4.3	3.0	
20	9-10	90	50	40	4.0	2.4	
21	0	130	40			1.0	
22	1-2	90	90			0.7	
23	5-6	90	50			0.6	
24	9-10	90	50			1.6	
Cycloheximide ($50mgkg^{-1}$)							
25	0	130	40	14	3.0	2.1	
26	1-2	90	50	14	5.3	2.0	
27	5-6	90	50	16	3.6	1.4	
28	9-10	90	50	16	3.1	1.0	

Blood pH and blood gas measurements (n=6) -:

$pH = 7.32 \pm 0.01$

$pCO_2 = 36.4 \pm 1.37$

$pO_2 = 256.2 \pm 3.61$

DOG 3 19kg, F

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
Control							
1	0	120	85	30	3.3	3.7	56
2	1-2	80	130	99	4.7	4.9	32
3	5-6	80	130	103	5.1	4.8	58
4	9-10	80	100	147	5.2	3.8	58
5	0	120	70	53	2.0	3.2	
6	1-2	80	110	147	2.9	5.2	
7	5-6	80	110	114	3.3	3.8	
8	9-10	80	90	155	4.6	3.0	
Captopril (25mg)							
9	0	120	60	18	3.6	4.0	272
10	1-2	80	75	77	7.0	7.0	82
11	5-6	80	75	58	9.4	2.3	288
12	9-10	80	60	65	5.7	2.7	192
13	0	120	50	12	9.3	2.8	
14	1-2	80	70	52	6.7	3.2	
15	5-6	80	70	73	9.5	3.0	
16	9-10	80	60		3.7	2.4	
AII ($5ngmin^{-1}$)							
17	0	120	60	15	2.3	1.2	
18	1-2	80	90	53	3.0	6.0	
19	5-6	80	90	96	3.8	4.5	
20	9-10	80	85	36	3.0	4.4	
21	0	120	60	58	3.0	3.0	
22	1-2	80	85	160	3.2	4.0	
23	5-6	80	85	120	3.5	3.5	
24	9-10	80	60	120	3.0	2.6	
Cycloheximide ($50mgkg^{-1}$)							
25	0	120	70	1		5.4	
26	1-2	80	110	3		6.5	
27	5-6	80	110	2		5.3	
28	9-10	80	100	0		4.0	

Blood pH and blood gas measurements (n=9) -:

pH = 7.43 ± 0.02

pCO_2 = 32.9 ± 0.90

pO_2 = 150.0 ± 16.7

DOG 4 20.5kg, M

S	T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
DRUG							
Control							
1	0	120	70	8	2.0	2.3	13
2	1-2	80	130	16	2.0	4.8	34
3	5-6	80	130	24	2.1	3.5	33
4	9-10	80	110	24	2.2	2.9	17
Captopril (25mg)							
5	0	120	60	12	5.7	3.5	44
6	1-2	80	90	8	5.0	5.8	43
7	5-6	80	90	8	6.5	4.5	63
8	9-10	80	70	10	3.2	4.0	43
9	0	120	65			2.9	34
10	1-2	80	110			6.0	35
11	5-6	80	110			4.2	26
12	9-10	80	70			2.8	30
AII ($5ngmin^{-1}$)							
13	0	120	70	12	1.0	2.0	
14	1-2	80	120	14	1.6	3.8	
15	5-6	80	120	18	3.8	2.8	
16	9-10	80	100	22	3.0	2.3	
17	0	120	55			1.5	
18	1-2	80	110			1.0	
19	5-6	80	110			0.8	
20	9-10	80	100			0.7	
Cycloheximide ($50mgkg^{-1}$)							
21	0	120	50	18	3.3	1.2	
22	1-2	80	110	8		7.2	
23	5-6	80	110	0		3.5	
24	9-10	80	110	0		2.2	

Blood pH and blood gas measurements (n=9) -:

pH = 7.35 ± 0.02

pCO_2 = 45.1 ± 0.90

pO_2 = 223.9 ± 1.71

DOG 5 26kg, M

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
Control							
1	0	140	85	35	2.2	2.9	33
2	1-2	100	170	66	2.7	3.7	12
3	5-6	100	170	65	2.3	3.8	17
4	9-10	100	170	78	2.6	3.8	17
5	0	140	70			2.9	39
6	1-2	100	110			3.6	29
7	5-6	100	110			3.5	31
8	9-10	100	110			3.0	17
Captopril (25mg)							
9	0	125	40	34	3.0	2.7	38
10	1-2	85	65	38	3.5	3.2	45
11	5-6	85	65	58	2.9	3.1	41
12	9-10	85	65	58	2.9	2.9	29
13	0	125	55				
14	1-2	85	120				
15	5-6	85	120				
16	9-10	85	120				
AII ($5ngmin^{-1}$)							
17	0	125	45	34	3.0	1.9	
18	1-2	85	60	35	1.7	2.5	
19	5-6	85	60	90	2.6	2.3	
20	9-10	85	60	97	2.1	2.2	
21	0	125	35			1.5	
22	1-2	85	70			2.1	
23	5-6	85	70			2.6	
24	9-10	85	70			2.9	
Cycloheximide ($50mgkg^{-1}$)							
25	0	125	45	11	4.5	3.6	
26	1-2	85	75	16	4.5	6.0	
27	5-6	85	75	11	4.3	2.2	
28	9-10	85	75	7		2.9	

Blood pH and blood gas measurements (n=7) -:

pH = 7.43 ± 0.01

pCO_2 = 36.6 ± 0.80

pO_2 = 185.0 ± 24.1

DOG 6. 14kg, F

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
	Control						
1	0	110	60	26	2.6	2.5	37
2	1-2	70	140	54	3.1	5.3	11
3	5-6	70	140	66	2.2	4.9	10
4	9-10	70	140	92	4.5	4.1	17
	Captopril (25mg)						
5	0	110	70	32	2.2	4.2	50
6	1-2	70	145	38	3.1	7.2	56
7	5-6	70	145	24	1.5	6.0	69
8	9-10	70	145	26	2.0	4.8	52
9	0	110		24	2.2	4.1	43
10	1-2	70		38	2.1	6.0	58
11	5-6	70		36	1.5	2.0	29
12	9-10	70		40	2.2	2.0	32

Blood pH and blood gas measurements (n=8) -:

pH = 7.41 ± 0.05

pCO_2 = 31.0 ± 3.40

pO_2 = 314.6 ± 11.0

DOG 7. 28kg, F

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
Control							
1	0	110	100	15	8.2	3.8	4
2	1-2	70	195	35	6.8	6.1	4
3	5-6	70	195	28	5.0	5.5	4
4	9-10	70	195	19	4.5	5.5	6
5	0	110	110	14	6.0	4.0	6
6	1-2	70	200	24	7.0	6.5	14
7	5-6	70	200	13	5.0	5.7	13
8	9-10	70	200	29	5.0	5.7	9
Captopril (25mg)							
9	0	110	90	10	2.3	6.0	33
10	1-2	70	170	19	4.0	8.0	32
11	5-6	70	170	15	5.1	7.4	42
12	9-10	70	170	13	4.0	6.8	34
13	0	110	90	14	2.8	4.8	
14	1-2	70	170	14	4.0	7.2	
15	5-6	70	170	14	5.0	6.1	
16	9-10	70	170	16	2.5	5.5	
ACTH (100µg)							
17	0	110	90			3.9	
18	1-2	70	175			2.6	
19	5-6	70	175			2.2	
20	9-10	70	175			2.1	
21	0	110	95			4.4	
22	1-2	70	170			3.5	
23	5-6	70	170			2.8	
24	9-10	70	150			2.6	

Blood pH and blood gas measurements (n=9) -:

pH = 7.37 ± 0.01

pCO_2 = 36.7 ± 1.66

pO_2 = 203.9 ± 5.1

DOG 8. 22kg, M

S	DRUG	T +BRS	CPP	SBP	CA	Ratio A : NA	BF
	Control						
1		0	140	90	78	5.2	2.1
2		1-2	100	150	217	3.1	3.8
3		5-6	100	150	203	4.0	6.2
4		9-10	100	95	169	2.4	3.0
5		0	140	80			1.9
6		1-2	100	120			3.0
7		5-6	100	120			3.4
8		9-10	100	100			3.1
	Captopril (25mg)						
9		0	120	75	67	2.9	1.5
10		1-2	80	125	58	2.9	4.2
11		5-6	80	125	98	3.2	3.2
12		9-10	80	125	63	4.6	2.5
13		0	120	70	48	2.9	3.0
14		1-2	80	120			3.5
15		5-6	80	120			2.8
16		9-10	80	120			4.3
	ACTH (100µg)						
17	+10 min.	0	120		114	2.7	4.0
18		0	120	60	34	3.8	3.4
19		1-2	80	90	77	3.8	3.9
20		5-6	80	90	62	3.3	3.4
21		9-10	80	90	77	2.9	3.4
22		0	120	80	34	3.8	4.0
23		1-2	80	135	48	2.9	4.0
24		5-6	80	135	67	2.9	4.4
25		9-10	80	135	48	1.8	4.0
26	+50 min.	0	120		55	4.0	3.5

Blood pH and blood gas measurements (n=7) -:

pH = 7.32 ± 0.02

pCO_2 = 43.7 ± 0.60

pO_2 = 242.1 ± 14.5

DOG 9. 16kg, F

S	DRUG	T +BRS	CPP	SBP	CA	Ratio A : NA	BF
	Control						
1		0	110	90	61	3.6	3.3
2		1-2	70	140	153	4.2	4.9
3		5-6	70	140	137	3.1	2.9
4		9-10	70	110	89	3.3	3.0
	Captopril (25mg)						
5		0	110	70	24	4.9	2.9
6		1-2	70	100	40	2.7	8.8
7		5-6	70	100	28	2.0	5.3
8		9-10	70	100	29	2.5	5.8
9		0	110	70	21	4.6	2.7
10		1-2	70	120			7.1
11		5-6	70	120	55	2.9	5.2
12		9-10	70	900	57	3.5	5.6
	ACTH (100µg)						
13	+10 min.	0	110		41	3.5	4.0
14		0	110	70	44	3.2	3.8
15		1-2	70	120	67	4.4	9.0
16		5-6	70	120	85	3.6	5.3
17		9-10	70	110	70	3.1	6.1
18	+30 min.	0	110		57	2.6	5.0
19		0	110	70	47	5.1	4.7
20		1-2	70	110	50	3.3	7.4
21		5-6	70	110	67	2.1	6.7
22		9-10	70	110	61	2.4	6.8

Blood pH and blood gas measurements (n=7) -:

pH = 7.39 ± 0.02

pCO_2 = 35.6 ± 1.00

pO_2 = 203.9 ± 30.6

DOG 10. 22kg, F

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF
Control						
1	0	140	75	24	8.3	2.5
2	1-2	100	120	373	9.4	3.5
3	5-6	100	120	687	4.6	3.8
4	9-10	100	120	93	8.4	3.0
5	0	140	75	23	4.1	1.7
6	1-2	100	120	82	7.5	2.2
7	5-6	100	120	82	6.5	1.8
8	9-10	100	120			1.6
Cycloheximide ($50 mg kg^{-1}$)						
9	0	140	70	46	5.9	1.6
10	1-2	100	110	80	4.1	4.0
11	5-6	100	110	68	4.6	3.2
12	9-10	100	110	66	9.8	2.5
13	0	140	70			1.0
14	1-2	100	110	16	5.0	3.9
15	5-6	100	110	50	3.8	4.2
16	9-10	100	110			4.0

Blood pH and blood gas measurements (n=7) -:

pH = 7.39 ± 0.01

pCO_2 = 36.4 ± 2.20

pO_2 = 208.6 ± 19.5

DOG 11. 23kg, F

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF
	Control					
1	0	140	60	23	3.3	3.3
2	1-2	100	100			4.9
3	5-6	100	100	26	4.4	2.9
4	9-10	100	100	31	3.8	3.0
	Captopril (25mg)					
5	0	130	50	49	2.1	2.9
6	1-2	90	90	31	1.9	8.8
7	5-6	90	90	22	2.4	5.3
8	9-10	90	80	27	1.8	4.0
9	0	130	50	68	3.5	2.7
10	1-2	90	80			7.1
11	5-6	90	80	39	6.3	5.2
12	9-10	90	60	74	4.0	5.6
	ACTH (100µg)					
13	0	130	55	51	3.3	3.8
14	1-2	90	70	72	2.8	9.0
15	5-6	90	70	37	2.0	5.3
16	9-10	90	70	64	2.7	6.1
17	0	130	55	30	3.7	4.7
18	1-2	90	80	57	5.5	7.4
19	5-6	90	80			6.7
20	9-10	90	65	39	3.4	6.8

Blood pH and blood gas measurements (n=5) -:

pH = 7.46 ± 0.02

pCO_2 = 34.6 ± 2.00

pO_2 = 190.8 ± 25.3

DOG 12. 20.5kg, M

S	DRUG	T +BRS	CPP	SBP	CA	Ratio A : NA	BF
	Control						
1		0	140	110	57	5.3	4.9
2		1-2	100	160	122	7.1	6.5
3		5-6	100	160	107	7.9	5.5
4		9-10	100	115	103	7.6	5.0
	Captopril (25mg)						
5		0	140	95	48	5.9	5.6
6		1-2	100	110	73	6.3	6.0
7		5-6	100	110	63	5.3	5.2
8		9-10	100	110	96	6.4	5.5
9		0	140	95			4.5
	ACTH (100ug)						
10	+10 min	0	140				4.5
11	+20 min	0	140				4.3
12	+30 min	0	140	80	46	5.6	4.3
13		1-2	100	110	78	2.7	5.0
14		5-6	100	110	88	3.4	4.5
15		9-10	100	110	68	3.5	8.0
16	+40 min	0	140				3.5
17		0	140	70	38	3.8	7.1
18		1-2	100	95	61	1.9	4.7
19		5-6	100	95	66	2.9	8.3
20		9-10	100	95	65	2.8	8.3
21	+50 min	0	140				8.3

Blood pH and blood gas measurements (n=5) -:

pH = 7.19 ± 0.005

pCO_2 = 42.2 ± 3.80

pO_2 = 218.0 ± 34.3

DOG 13. 25kg, M

S	DRUG	Cortisol conc.	Cortico conc.	BF
	Control			
1		4.63	2.94	6.2
2		4.56	2.16	6.4
	Captopril (25mg)			
3		3.38	1.96	8.8
4		2.84	2.14	7.6
	AII ($5ngmin^{-1}$)			
5		6.39	3.44	4.0
6		6.73	4.06	3.2

Blood pH and blood gas measurements (n=6) -:

pH = 7.25 ± 0.02

pCO_2 = 50.6 ± 2.05

pO_2 = 130.7 ± 29.3

(All catecholamine samples lost)

DOG 14 (+19). 18kg, F

S	DRUG	Test	PPS	SBP	CA	Ratio A : Na	BF	Cortisol conc.	Cortico. conc.
	Control								
1		C		90	152	4.6	1.6		
2		+ S	2.5	105	122	4.1	0.8		
3							2.1	8.63	4.59
4		C		90	216	3.5	2.4		
5		+ S	5.0	110	494	3.8	2.9		
6							2.0	10.4	5.31
7		C		100	225	3.3	2.1		
8		+ S	10.0	110	451	3.3	2.2		
9							2.7	9.99	4.31
10		C		70	304	3.3	2.3		
11		+ S	20.0	80	477	3.3	2.8		
	Captopril (25mg)								
12							6.1	5.89	2.10
13		C		45	196	3.1	2.9		
14		+ S	2.5	50	83	2.8	1.3		
15							3.9	8.27	2.87
16		C		60	216	2.2	6.2		
17		+ S	5.0	65	144	2.9	5.5		
18		C		85	99	2.8	3.8		
19		+ S	10.0	85	82	2.9	3.2		
20		C		100	91	2.6	3.4		
21		+ S	20.0	100	101	2.6	3.7		
	AII ($5ngmin^{-1}$)								
22							2.9	10.7	2.66
23		C		90			3.5		
24		+ S	2.5	90			4.0		
25							4.5	6.12	1.83
26		C		75			5.0		
27		+ S	5.0	75			5.2		
28		C		100			5.1		
29		+ S	10.0	100			5.0		
30		C		90	101	1.2	5.1		
31		+ S	20.0	90	182	1.2	5.7		

Blood pH and blood gas measurements (n=9) -:

pH = 7.42 ± 0.02

pCO_2 = 37.8 ± 1.60

pO_2 = 243.8 ± 24.1

DOG 15 (+20). 26kg, M

S	DRUG	Test	PPS	SBP	CA	Ratio A : Na	BF	Cortisol conc.	Cortico. conc.
	Control								
1		C		75	212	4.7	3.5		
2		+ S	2.5	85	250	4.8	3.5		
3							1.7	5.39	2.44
4		C		60	353	4.9	2.8		
5		+ S	5.0	60	392	5.0	2.7		
6							2.5	4.74	2.36
7		C		60	439	4.9	2.5		
8		+ S	10.0	60	523	4.8	2.5		
9		C		60	514	4.7	2.6		
10		+ S	20.0	60	530	4.6	2.8		
	Captopril (25mg)								
11							3.3	2.92	1.09
12		C		55	239	4.1	2.9		
13		+ S	2.5	55	240	4.1	3.7		
14							2.8	2.28	0.84
15		C		55	510	4.7	3.5		
16		+ S	5.0	55	446	4.0	2.9		
17		C		60	369	3.9	3.3		
18		+ S	10.0	60	350	3.8	3.1		
19		C		45	440	3.7	3.0		
20		+ S	20.0	45	407	3.7	2.7		

Blood pH and blood gas measurements (n=8) -:

pH = 7.30 ± 0.01

pCO_2 = 42.9 ± 1.00

pO_2 = 151.1 ± 15.7

DOG 16. 22kg, M

S	DRUG Test	PPS	SBP	CA	Ratio A : Na	BF	Cortico. conc.
	Control						
1	C		110			5.8	
2	+ S	2.5	110			4.7	
3						5.0	2.71
4	C		105			7.8	
5	+ S	5.0	110			7.9	
6						6.9	1.75
7	C		105			7.6	
8	+ S	10.0	120			7.2	
9	C		110			7.5	
10	+ S	20.0	130			7.5	
	Captopril (25mg)						
11						5.4	1.77
12	C		100			6.6	
13	+ S	2.5	100			5.2	
14						4.4	2.29
15	C		100			5.0	
16	+ S	5.0	105			4.7	
17	C		90			3.4	
18	+ S	10.0	100			3.3	
19	C		85			4.8	
20	+ S	20.0	100			5.2	
	AII ($5ngmin^{-1}$)						
21						2.3	3.13
22	C		85			3.3	
23	+ S	2.5	85			3.5	
24						3.5	1.74
25	C		85			4.1	
26	+ S	5.0	85			4.0	
27	C		85			5.1	
28	+ S	10.0	90			5.5	
29	C		80			4.9	
30	+ S	20.0	85			5.9	

Blood pH and blood gas measurements (n=8) -:
pH = 7.41 ± 0.01
pCO_2 = 40.6 ± 1.30
pO_2 = 182.1 ± 9.60
(All catecholamine samples lost)

DOG 17. 21kg, F

S	DRUG	Test	PPS	SBP	CA	Ratio A : Na	BF	Cortisol conc.	Cortico. conc.
	Control								
1		C		105			6.8		
2		+ S	2.5	140			7.2		
3							6.2	2.61	2.74
4		C		105			6.6		
5		+ S	5.0	140			7.3		
6							6.1	2.10	1.72
7		C		110			6.4		
8		+ S	10.0	140			7.0		
9		C		105			6.0		
10		+ S	20.0	135			7.0		
	Captopril (25mg)								
11							5.0	3.90	2.25
12		C		100			5.5		
13		+ S	2.5	120			5.8		
14							3.1	5.34	1.81
15		C		110			3.5		
16		+ S	5.0	135			3.4		
17		C		115			2.7		
18		+ S	10.0	140			2.7		
19		C		115			2.5		
20		+ S	20.0	140			2.4		
	AII ($5ngmin^{-1}$)								
21		C		100			2.5		
22		+ S	2.5	105			3.0		
23							2.4	5.05	3.08
24		C		110			2.8		
25		+ S	5.0	115			2.7		
26							2.4	8.09	1.79
27		C		100			3.2		
28		+ S	10.0	110			3.4		
29		C		100			3.5		
30		+ S	20.0	115			3.2		

Blood pH and blood gas measurements (n=6) -:

pH = 7.37 ± 0.02

pCO_2 = 42.5 ± 2.50

pO_2 = 191.8 ± 16.7

(All catecholamine samples lost)

DOG 18. 30kg, F

S	DRUG Test	PPS	SBP	CA	Ratio A : Na	BF	Cortisol conc.	Cortico. conc.
	Control							
1	C		135			2.5		
2	+ S	2.5	135			2.5		
3						2.5	3.99	3.67
4	C		120			2.0		
5	+ S	5.0	120			2.0		
6						1.7	4.71	3.68
7	C		120			2.1		
8	+ S	10.0	120			1.9		
9	C		100			2.0		
10	+ S	20.0	100			2.0		
	Captopril (25mg)							
11						3.0	4.73	3.41
12	C		100			3.0		
13	+ S	2.5	100			3.0		
14						2.5	4.94	2.74
15	C		100			2.7		
16	+ S	5.0	100			2.8		
17	C		95			2.9		
18	+ S	10.0	95			1.0		
19	C		85			1.6		
20	+ S	20.0	85			2.4		
	AII ($5ngmin^{-1}$)							
21	C		90			2.5		
22	+ S	2.5	90			2.7		
23						2.5	2.40	1.26
24	C		70			2.6		
25	+ S	5.0	70			2.8		
26						2.5	3.11	1.10
27	C		65			2.9		
28	+ S	10.0	65			2.7		
29	C		35			1.0		
30	+ S	20.0	35			0.6		

Blood pH and blood gas measurements (n=8) -:

pH = 7.39 ± 0.02

pCO_2 = 38.5 ± 0.80

pO_2 = 129.4 ± 35.5

(All catecholamine samples lost)

DOG 19 (+14). 18kg, F

S	DRUG	Test	PPS	SBP	CA	Ratio A : Na	BF	Cortisol conc.	Cortico. conc.
	Control								
1		C		90	152	4.6	1.6		
2		+ S	2.5	105	122	4.1	0.8		
3							2.1	8.63	4.59
4		C		90	216	3.5	2.4		
5		+ S	5.0	110	494	3.8	2.9		
6							2.0	10.4	5.31
7		C		100	225	3.3	2.1		
8		+ S	10.0	110	451	3.3	2.2		
9							2.7	9.99	4.31
10		C		70	304	3.3	2.3		
11		+ S	20.0	80	477	3.3	2.8		
	Captopril (25mg)								
12							6.1	5.89	2.10
13		C		45	196	3.1	2.9		
14		+ S	2.5	50	83	2.8	1.3		
15							3.9	8.27	2.87
16		C		60	216	2.2	6.2		
17		+ S	5.0	65	144	2.9	5.5		
18		C		85	99	2.8	3.8		
19		+ S	10.0	85	82	2.9	3.2		
20		C		100	91	2.6	3.4		
21		+ S	20.0	100	101	2.6	3.7		
	AII ($5ngmin^{-1}$)								
22							2.9	10.7	2.66
23		C		90			3.5		
24		+ S	2.5	90			4.0		
25							4.5	6.12	1.83
26		C		75			5.0		
27		+ S	5.0	75			5.2		
28		C		100			5.1		
29		+ S	10.0	100			5.0		
30		C		90	101	1.2	5.1		
31		+ S	20.0	90	182	1.2	5.7		

Blood pH and blood gas measurements (n=9) -:

pH = 7.42 ± 0.02

pCO_2 = 37.8 ± 1.60

pO_2 = 243.8 ± 24.1

DOG 20 (+15). 26kg, M

S	DRUG Test	PPS	SBP	CA	Ratio A : Na	BF	Cortisol conc.	Cortico. conc.
	Control							
1	C		75	212	4.7	3.5		
2	+ S	2.5	85	250	4.8	3.5		
3						1.7	5.39	2.44
4	C		60	353	4.9	2.8		
5	+ S	5.0	60	392	5.0	2.7		
6						2.5	4.74	2.36
7	C		60	439	4.9	2.5		
8	+ S	10.0	60	523	4.8	2.5		
9	C		60	514	4.7	2.6		
10	+ S	20.0	60	530	4.6	2.8		
	Captopril (25mg)							
11						3.3	2.92	1.09
12	C		55	239	4.1	2.9		
13	+ S	2.5	55	240	4.1	3.7		
14						2.8	2.28	0.84
15	C		55	510	4.7	3.5		
16	+ S	5.0	55	446	4.0	2.9		
17	C		60	369	3.9	3.3		
18	+ S	10.0	60	350	3.8	3.1		
19	C		45	440	3.7	3.0		
20	+ S	20.0	45	407	3.7	2.7		

Blood pH and blood gas measurements (n=8) -:

pH = 7.30 ± 0.01

pCO_2 = 42.9 ± 1.00

pO_2 = 151.1 ± 15.7

DOG 21. 28kg, M

S	DRUG Test	PPS	SBP	CA	Ratio A : Na	BF
	Control					
1	C		105	11	2.7	3.5
2	+ S	2.5	110	24	3.8	3.6
3	C		110	6	5.0	3.0
4	+ S	5.0	130	54	4.4	4.0
5	C		110			5.2
6	+ S	10.0	130			6.2
7	C		110	17	3.3	2.7
8	+ S	20.0	120	20	2.8	3.4
	Captopril (25mg)					
9	C		100	28	3.0	4.0
10	+ S	2.5	105	19	3.8	4.0
11	C		100	12	5.0	2.5
12	+ S	5.0	110	50	4.6	3.3
13	C		100	16	4.3	2.2
14	+ S	10.0	120	13	3.9	3.4
15	C		110	29	4.8	2.2
16	+ S	20.0	120	22	2.6	3.4
	AII ($5ngmin^{-1}$)					
17	C			28	3.7	2.0
18	+ S	2.5		26	4.2	1.9
19	C		115	24	5.0	2.0
20	+ S	5.0	120	47	4.9	2.2
21	C		100	31	4.2	2.5
22	+ S	10.0	120	11	3.9	2.8
23	C		110	48	5.0	2.7
24	+ S	20.0	120	186	2.8	3.5

Blood pH and blood gas measurements (n=6) -:

pH = 7.38 ± 0.02

pCO_2 = 39.0 ± 1.10

pO_2 = 165.8 ± 15.0

DOG 22. 22kg, M

S	DRUG Test	PPS	SBP	CA	Ratio A : Na	BF
	Control					
1	C		80	124	4.4	2.3
2	+ S	2.5	85	93	5.2	1.7
3	C		70	91	4.7	1.2
4	+ S	5.0	85	148	4.1	2.7
5	C		40	168	3.9	0.8
6	+ S	10.0	80	275	3.5	3.5
7	C		50	168	3.9	1.5
8	+ S	20.0	65	208	3.5	2.3
	Captopril (25mg)					
9	C		60	83	5.4	4.6
10	+ S	2.5	65	52	3.3	5.3
11	C		55	98	3.1	4.5
12	+ S	5.0	60	100	3.2	4.2
13	C		40	113	3.0	2.8
14	+ S	10.0	40	147	3.1	2.5
15	C		40	69	3.3	1.3
16	+ S	20.0	40	88	3.4	1.4
	AII ($5ngmin^{-1}$)					
17	C		40	139	3.1	2.7
18	+ S	2.5	40	118	2.7	2.4
19	C		40	177	2.7	3.0
20	+ S	5.0	40	250	2.5	3.0
21	C		25	262	2.9	1.4
22	+ S	10.0	25	330	3.1	1.2
23	C		35	285	3.0	2.0
24	+ S	20.0	35	308	3.0	2.2

Blood pH and blood gas measurements (n=8) -:

pH = 7.33 ± 0.03

pCO_2 = 43.3 ± 2.60

pO_2 = 202.8 ± 15.7

DOG 23. 25kg, F

S	DRUG Test	PPS	SBP	CA	Ratio A : Na	BF
	Control					
1	C		110	103	2.8	7.3
2	+ S	2.5	130	65	3.7	7.1
3	C		110	120	3.6	6.6
4	+ S	5.0	125	159	3.7	6.5
5	C		110	116	3.9	5.8
6	+ S	10.0	135	261	4.0	5.8
7	C		105	182	4.0	4.2
8	+ S	20.0	125	344	3.4	4.2
	Captopril (25mg)					
9	C		75			4.8
10	+ S	2.5	80			4.0
11	C		85	186	3.7	4.9
12	+ S	5.0	100	115	4.3	2.7
13	C		90	174	3.8	4.7
14	+ S	10.0	110	261	3.8	6.6
15	C		95	158	3.9	3.6
16	+ S	20.0	110	214	3.9	3.5
	AII ($5ngmin^{-1}$)					
17	C		85	378	4.4	2.1
18	+ S	2.5	85	467	4.0	2.8

Blood pH and blood gas measurements (n=5) -:

pH = 7.32 ± 0.03

pCO_2 = 44.0 ± 1.80

pO_2 = 237.5 ± 13.3

DOG 24. 25kg, M

S	DRUG Test	PPS	SBP	CA	Ratio A : Na	BF
	Control					
1	C		105	314	7.3	5.5
2	+ S	2.5	105	42	6.5	5.2
3	C		90	11	5.3	4.5
4	+ S	5.0	100	75	5.6	5.1
5	C		85	29	5.6	5.5
6	+ S	10.0	100	244	5.6	5.1
7	C		90	21	6.2	5.4
8	+ S	20.0	100	253	2.9	4.7
	Captopril (25mg)					
9	C		85	17	5.4	4.2
10	+ S	2.5	90	33	6.8	4.4
11	C		90	30	7.4	3.8
12	+ S	5.0	100	92	5.2	4.3
13	C		80	23	7.7	3.8
14	+ S	10.0	105	107	3.9	4.0
15	C		90	33	6.6	4.0
16	+ S	20.0	95	185	3.4	4.0
	AII ($5 ngmin^{-1}$)					
17	C		90	78	10.0	2.8
18	+ S	2.5	100	86	7.2	3.3
19	C		85	71	8.7	2.5
20	+ S	5.0	100	91	7.2	3.0
21	C		75	145	7.9	2.9
22	+ S	10.0	105	220	5.6	2.4
23	C		75	248	7.2	2.9
24	+ S	20.0	95	299	4.4	2.5

Blood pH and blood gas measurements (n=5) -:

pH = 7.37 ± 0.01

pCO_2 = 37.4 ± 0.70

pO_2 = 151.0 ± 7.90

DOG 25. 19kg, M

S	DRUG Test	SBP	CA	Ratio A : Na	BF
Control					
1	C	125	88	3.6	6.0
2	+ S	155	163	4.4	5.0
3	C	130	64	3.9	6.7
4	+ S	160	151	4.1	5.5
Captopril (25mg)					
5	C	110	64	4.2	7.6
6	+ S	155	116	4.2	6.5
7	C	125	74	4.2	
8	+ S	155	93	5.0	

Blood pH and blood gas measurements (n=5) -:

pH = 7.34 ± 0.02

pCO_2 = 41.6 ± 2.00

pO_2 = 214.8 ± 22.3

DOG 26. 18kg, F

S	DRUG Test	SBP	CA	Ratio A : Na	BF
Control					
1	C	120	58	5.3	2.4
2	+ S	135	72	6.9	2.2
3	C	115	60	5.9	2.5
4	+ S	120	73	6.7	2.5
Captopril (25mg)					
5	C	85	45	7.7	2.7
6	+ S	95	45	7.2	2.1
7	C	100	41	5.3	3.5
8	+ S	100	39	6.4	3.6
AII ($10ngmin^{-1}$)					
9	C	115	64	5.3	3.8
10	+ S	115	206	6.8	4.3
AII ($30ngmin^{-1}$)					
11	C	110	66	6.7	4.1
12	+ S	115	251	6.4	4.0
AII ($100ngmin^{-1}$)					
13	C	115	104	5.8	3.8
14	+ S	115	271	4.7	3.9

Blood pH and blood gas measurements (n=6) -:

pH = 7.40 ± 0.04

pCO_2 = 31.7 ± 1.30

pO_2 = 237.8 ± 15.1

DOG 27. 20kg, M

S	DRUG Test	SBP	CA	Ratio A : Na	BF
Control					
1	C	90	47	1.6	5.4
2	+ S	90	96	3.8	1.9
3	C				5.0
4	+ S				4.5
Captopril (25mg)					
5	C	70			6.0
6	+ S	70			6.5
7	C				5.5
8	+ S				5.5
AII ($10ngmin^{-1}$)					
9	C	70	302	3.1	5.5
10	+ S	70	489	3.9	5.3
AII ($30ngmin^{-1}$)					
11	C	65	306	4.3	3.2
12	+ S	65	358	5.0	3.2
AII ($100ngmin^{-1}$)					
13	C	55	472	4.0	2.7
14	+ S	55	750	4.2	2.7

Blood pH and blood gas measurements (n=3) -:

pH = 7.33 ± 0.02

pCO_2 = 35.7 ± 1.50

pO_2 = 238.3 ± 22.8

DOG 28. 26kg, M

S	DRUG Test	SBP	CA	Ratio A : Na	BF
Control					
1	C	105	23	3.3	7.0
2	+ S	115	106	2.6	6.2
3	C	105			6.2
4	+ S	115			5.7
Captopril (25mg)					
5	C	95	51	4.0	8.7
6	+ S	105	85	2.8	10.2
7	C	75	96	2.1	8.2
8	+ S	85	167	2.4	11.0
AII ($10ngmin^{-1}$)					
9	C	75	137	2.6	9.5
10	+ S	90	169	3.0	9.9
AII ($30ngmin^{-1}$)					
11	C	90	79	3.4	8.5
12	+ S	100	137	2.5	8.8
AII ($100ngmin^{-1}$)					
13	C	100	87	3.2	7.7
14	+ S	115	147	4.5	8.2

Blood pH and blood gas measurements (n=6) -:

pH = 7.30 ± 0.02

pCO_2 = 41.5 ± 3.20

pO_2 = 247.5 ± 15.9

DOG 29. 19kg, F

S	DRUG Test	SBP	CA	Ratio A : Na	BF	
	Control					
1	C	115	52	2.9	4.7	
2	+ S	125	92	5.7	4.5	
3	C	105	58	5.4	3.5	
4	+ S	120	197	8.1	3.7	
	Saralasin (10ugminkg^{-1})					
5	C	85	30	3.8	2.9	
6	+ S	95	49	4.7	1.9	
7	C	75	39	4.1	2.2	
8	+ S	90	67	4.0	3.1	
	Saralasin infusion off					
9	C	110	54	5.5	2.4	
10	+ S	125	86	8.2	3.0	
11	C	110	56	3.2	2.2	Cortisol
12	+ S	130	164	5.0	2.5	conc.
	Control					
13		100	52	5.1	2.3	1.49
14		100	58	2.7	1.6	2.29
	ACTH (100ug)					
15	+10 min	100	118	2.7	1.3	3.36
16	+20 min	90	178	4.7	1.2	3.70
17	+30 min	90	223	3.5	1.2	3.60

Blood pH and blood gas measurements (n=4) -:

pH = 7.23 ± 0.03

pCO_2 = 47.8 ± 6.60

pO_2 = 320.0 ± 46.7

DOG 30. 25kg, F

S	DRUG Test	SBP	CA	Ratio A : Na	BF
Control					
1	C	115	67	3.2	3.1
2	+ S	120	91	4.7	3.6
3	C	105	282	3.8	3.1
4	+ S	105	290	3.9	3.6
Saralasin (10ugminkg^{-1})					
5	C	90	262	3.7	3.0
6	+ S	90	133	3.8	3.5
Saralasin infusion off					
7	C	95	213	3.1	4.1
8	+ S	95	279	3.1	4.5
9	C	95	619	3.1	4.4
10	+ S	95	759	2.9	4.0

Blood pH and blood gas measurements (n=6) -:

pH = 7.29 ± 0.02

pCO_2 = 43.0 ± 1.00

pO_2 = 217.3 ± 35.7

DOG 31. 22kg, M

DRUG S	Test	SBP	CA	Ratio A : Na	BF
Control					
1	C	105	272	4.0	4.6
2	+ S	105	320	3.7	4.3
3	C	105	226	4.5	4.0
4	+ S	105	409	2.8	3.7
Saralasin (10ugminkg^{-1})					
5	C	80	202	3.5	2.9
6	+ S	80	260	3.4	2.9
7	C	90	239	3.9	3.3
8	+ S	90	236	3.7	3.0
Saralasin infusion off					
9	C	115	282	3.4	2.6
10	+ S	115	323	3.0	2.7
11	C	105	458	2.9	2.4
12	+ S	105	479	3.0	2.3

Blood pH and blood gas measurements (n=4) -:

pH = 7.30 ± 0.02

pCO_2 = 39.8 ± 2.50

pO_2 = 172.5 ± 26.2

DOG 32. 17kg, F

Diet = Salt loaded

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
	Control						
1	0	100	85	187	3.4	3.0	24.3
2	1-2	60	150	245	3.7	5.4	
3	5-6	60	150	240	3.9	3.6	
4	0	100	70	171	3.8	2.4	20.3
5	1-2	60	135	303	3.4	4.9	
6	5-6	60	135			3.2	
	Captopril (25mg)						
7	0	100	65	90	4.5	4.9	12.6
8	1-2	60	100	100	4.5	5.5	
9	5-6	60	100	90	4.4	4.7	
10	0	100	60	76	4.6	4.1	
11	1-2	60	110	160	6.2	5.0	
12	5-6	60	110	105	2.1	4.7	
	Naloxone ($0.3 mg min^{-1}$)						
13	0	100	120	157	3.7	4.9	
14	1-2	60	170	241	3.3	6.1	
15	5-6	60	170	355	3.6	5.3	
16	0	100	120				
17	1-2	60	170				
18	5-6	60	170				

Blood pH and blood gas measurements (n=6) -:

pH = 7.37 ± 0.01

pCO_2 = 39.4 ± 1.20

pO_2 = 192.0 ± 8.2

DOG 33. 21kg, M

Diet = Salt depleted

S	DRUG	T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
	Control							
1		0	110	140	17	4.5	3.5	20.6
2		1-2	70	200	34	6.0	5.5	
3		5-6	70	200	49	4.3	4.7	
4		0	110	95				10.8
5		1-2	70	165				
6		5-6	70	165				
	Naloxone ($0.3 mg min^{-1}$)							
7		0	110	90	137	10.1	4.3	
8		1-2	70	130	278	8.2	5.4	
9		5-6	70	130	231	6.4	3.9	
	Naloxone infusion off							
10		0	110	45	59	6.6	1.4	
11		1-2	70	85	253	7.6	3.6	
12		5-6	70	85	135	8.2	3.2	
	Captopril (25mg)							
13		0	110	45	82	6.1	3.4	21.1
14		1-2	70		96	8.6	3.5	
15		5-6	70		143	4.3	5.0	
16		0	110					24.6
	Naloxone ($0.3 mg min^{-1}$)							
17		0	110	35	74	9.8	2.1	
18		1-2	70	50	151	12.5	3.8	
19		5-6	70	50	135	10.2	3.3	
	Naloxone infusion off							
20		0	110	55	75	6.0	3.7	
21		1-2	70	65	125	9.3	5.0	
22		5-6	70	65	84	9.0	3.3	

Blood pH and blood gas measurements (n=6) -:

pH = 7.31 ± 0.01

pCO_2 = 40.5 ± 2.70

pO_2 = 180.0 ± 11.8

DOG 34. 17kg, F

Diet = Salt loaded

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
Control							
1	0	110	65	8	3.3	2.1	14.3
2	1-2	70	100	34	3.7	3.0	
3	5-6	70	100	39	3.8	2.7	
4	0	110	60	68	3.8	2.6	10.0
5	1-2	70	120	144	4.9	4.4	
6	5-6	70	120				
Naloxone ($0.3 mg min^{-1}$)							
7	0	110	100	393	5.01	4.0	
8	1-2	70	150	499	4.5	5.4	
9	5-6	70	150	494	5.4	4.7	
Naloxone infusion off							
10	0	110	75	334	4.4	2.8	
11	1-2	70	120	462	4.3	6.6	
12	5-6	70	120	493	5.6	3.4	
Captopril (25mg)							
13	0	110	70	202	4.3	5.9	39.4
14	1-2	70	90	276	6.5	6.6	
15	5-6	70	90	290	4.9	6.2	
16	0	110					20.0
Naloxone ($0.3 mg min^{-1}$)							
17	0	110	70	114	5.4	3.6	
18	1-2	70	115	310	3.8	4.6	
19	5-6	70	115	131	4.7	3.0	
Naloxone infusion off							
20	0	110	90	261	4.9	4.7	
21	1-2	70	115	323	2.1	5.6	
22	5-6	70	115	281	6.5	2.3	

Blood pH and blood gas measurements (n=6) -:

pH = 7.41 ± 0.01

pCO_2 = 36.0 ± 1.60

pO_2 = 174.2 ± 11.1

DOG 35. 22kg, M

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF
	Control					
1	0	110		20	6.7	2.5
2	1-2	70		54	3.5	4.4
3	5-6	70		25	6.5	3.2
4	0	110		34	5.1	2.2
5	1-2	70		140	6.7	3.5
6	5-6	70		101	6.1	2.5
	Naloxone ($0.3 mg min^{-1}$)					
7	0	110		77	5.9	2.3
8	1-2	70		370	7.7	3.4
9	5-6	70		146	6.4	2.6
	Naloxone infusion off + Captopril (25mg)					
10	0	110		89	8.0	3.2
11	1-2	70		196	10.8	5.0
12	5-6	70		132	8.5	4.0
	Naloxone ($0.3 mg min^{-1}$)					
13	0	110		64	2.9	3.4
14	1-2	70		122	5.2	4.1
15	5-6	70		70	6.3	3.6
	Naloxone infusion off					
16	0	110		107	6.4	3.4
17	1-2	70		132	7.2	3.5
18	5-6	70		127	4.3	3.3

Blood pH and blood gas measurements (n=5) -:

pH = 7.33 ± 0.03

pCO_2 = 39.3 ± 2.50

pO_2 = 241.3 ± 16.3

DOG 36. 23kg, M

DRUG S	Test	SBP	CA	Ratio A : Na	BF
Control					
1	C	125			
2	+ S	135			
3	C	115	2	3.0	0.2
4	+ S	135	7	3.3	0.4
Naloxone ($0.3 mg min^{-1}$)					
5	C	140	6	10.5	0.9
6	+ S	160	28	6.3	0.6
7	C	135	10	9.3	0.7
8	+ S	165	151	6.1	1.8
Naloxone infusion off + Captopril (25mg)					
9	C	100	34	10.8	0.4
10	+ S	120	32	9.2	0.5
11	C	100	43	9.6	0.5
12	+ S	120	21	7.4	0.5

Blood pH and blood gas measurements (n=4) -:

pH = 7.42 ± 0.05

pCO_2 = 34.0 ± 2.00

pO_2 = 157.5 ± 4.80

DOG 37. 22kg, M

DRUG S	Test	SBP	CA	Ratio A : Na	BF
Control					
1	C	115	45	3.3	6.6
2	+ S	120	96	4.9	5.6
3	C	115	26	3.3	7.4
4	+ S	125	120	4.8	8.4
Naloxone ($0.3 mg min^{-1}$)					
5	C	120	187	4.8	7.4
6	+ S	135	314	4.5	5.6
7	C	100	406	4.1	6.8
8	+ S	120	584	3.9	8.0
Naloxone infusion off + Captopril (25mg)					
9	C	75	49	3.0	6.6
10	+ S	85	70	3.9	6.6
11	C	70	61	1.5	5.6
12	+ S	80	67	2.2	6.0
Naloxone ($0.3 mg min^{-1}$)					
13	C	70	72	2.1	4.3
14	+ S	80	113	3.2	4.5
15	C	70			
16	+ S	80			

Blood pH and blood gas measurements (n=8) -:

$pH = 7.31 \pm 0.03$

$pCO_2 = 43.3 \pm 3.10$

$pO_2 = 159.1 \pm 23.8$

DOG 38. 24kg, M

Diet = Salt depleted

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
	Control						
1	0	125	90	5	3.5	2.4	20.6
2	1-2	85	135	33	3.5	4.0	
3	5-6	85	135	16	3.6	4.2	
4	0	125	80				10.8
5	1-2	85	140				
6	5-6	85	140				
	Captopril (25mg)						
7	0	125	85	4		2.5	2.1
8	1-2	85	120	20	3.5	3.1	
9	5-6	85	120	14	3.0	2.8	
10	0	125	75				24.6
11	1-2	85	140				
12	5-6	85	140				

Blood pH and blood gas measurements (n=7) -:

pH = 7.36 ± 0.02

pCO_2 = 36.3 ± 1.50

pO_2 = 371.2 ± 14.7

DOG 39. 25kg, F

Diet = Salt depleted

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
	Control						
1	0	110	110	96	7.9	1.6	41.5
2	1-2	70	170	234	7.4	5.1	
3	5-6	70	170	178	6.9	3.4	
4	0	110	95				40.8
5	1-2	70	160				
6	5-6	70	160				
	Captopril (25mg)						
7	0	110	60	499	6.7	3.4	45.3
8	1-2	70	110	1081	5.7	4.7	
9	5-6	70	110	292	6.0	3.6	
10	0						26.3

Blood pH and blood gas measurements (n=5) -:

pH = 7.24 ± 0.02

pCO_2 = 46.8 ± 3.90

pO_2 = 185.0 ± 8.7

DOG 40. 16kg, F

Diet = Salt loaded

S	DRUG	T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
	Control							
1		0	115	120			4.7	1.90
2		1-2	75	215			7.8	
3		5-6	75	215			7.2	
4		0	115	105			4.5	4.00
5		1-2	75	205			4.3	
6		5-6	75	205			3.8	
	Captopril (25mg)							
7		0	115	100			8.0	16.0
8		1-2	75	180			6.0	
9		5-6	75	180			5.0	
10		0	115	90			7.1	11.7
11		1-2	75	180			4.9	
12		5-6	75	180			4.5	

Blood pH and blood gas measurements (n=5) -:

pH = 7.40 ± 0.03

pCO_2 = 36.6 ± 1.60

pO_2 = 335.0 ± 8.5

(All catecholamine samples lost)

DOG 41. 25kg, M

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
	Control						
1	0	120	130	65	4.4	6.1	28.2
2	1-2	80	180	115	4.8	7.1	
3	5-6	80	180	157	2.7	6.4	
	Indomethacin ($5mgkg^{-1}$)						
4	0	120	135	45	4.2	3.6	14.8
5	1-2	80	180	37	5.3	4.1	
6	5-6	80	180	27	3.6	3.7	
7	0	120	135	55	3.1	3.8	7.6
8	1-2	80	180	80	4.1	3.9	
9	5-6	80	180	105	4.7	3.9	
	Captopril (25mg) (+ Indomethacin)						
10	0	120	115	15	2.2	3.5	14.3
11	1-2	80	165	17	2.0	4.1	
12	5-6	80	165	27	3.0	4.0	

Blood pH and blood gas measurements (n=4) -:

pH = 7.33 ± 0.03

pCO_2 = 38.0 ± 1.90

pO_2 = 169.5 ± 3.1

DOG 42. 38kg, M

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
	Control						
1	0	110	120	117	3.7	4.2	7.60
2	1-2	70	160	387	2.6	4.4	
3	5-6	70	160	198	2.9	4.5	
	Indomethacin (5mgkg^{-1})						
4	0	110	85	213	2.9	1.9	17.5
5	1-2	70	150	291	2.4	3.3	
6	5-6	70	150	251	2.6	2.0	
7	0	110	70	107	3.0	1.0	28.2
8	1-2	70	125	288	2.1	2.8	
9	5-6	70	125	279	2.1	2.0	
	Captopril (25mg) (+ Indomethacin)						
10	0	110	65	152	2.3	3.5	28.2
11	1-2	70	80	191	2.1	4.0	
12	5-6	70	80	128	2.1	3.4	

Blood pH and blood gas measurements (n=5) -:

pH = 7.34 ± 0.01

pCO_2 = 38.3 ± 2.10

pO_2 = 185.0 ± 55.2

DOG 43. 22kg, M

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
	Control						
1	0	120	155	162	4.6	5.0	21.5
2	1-2	80	220	320	3.6	6.0	
3	5-6	80	220	202	3.1	4.9	
	Indomethacin ($5mgkg^{-1}$)						
4	0	120	140	60	6.5	3.2	25.1
5	1-2	80	200	60	4.5	4.1	
6	5-6	80	200	79	3.9	4.2	
7	0	120	120	59	4.9	2.9	24.2
8	1-2	80	200	118	4.9	4.0	
9	5-6	80	200	137	3.3	3.7	
	Captopril (25mg) (+ Indomethacin)						
10	0	120	105	52	2.5	4.6	38.1
11	1-2	80	190	87	5.2	6.5	
12	5-6	80	190	668	3.7	6.4	

Blood pH and blood gas measurements (n=4) -:

pH = 7.30 ± 0.04

pCO_2 = 39.3 ± 2.10

pO_2 = 286.3 ± 12.5

DOG 44. 25kg, M

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
	Control						
1	0	120	80	31	3.7	3.0	13.4
2	1-2	80	125	90	5.8	4.2	
3	5-6	80	125	138	3.5	3.8	
	Indomethacin ($5mgkg^{-1}$)						
4	0	120	70	10	3.7	1.5	15.7
5	1-2	80	145	70	3.1	2.4	
6	5-6	80	145	71	3.7	2.3	
7	0	120	70	40	4.1	1.2	11.2
8	1-2	80	135	99	4.9	1.8	
9	5-6	80	135	76	5.0	1.9	
	Captopril (25mg) (+ Indomethacin)						
10	0	120	70				14.3
11	1-2	80	115				
12	5-6	80	115				

Blood pH and blood gas measurements (n=5) -:

pH = 7.40 ± 0.03

pCO_2 = 36.5 ± 1.60

pO_2 = 235.0 ± 15.0

DOG 45. 26kg, M

DRUG S	Test	SBP	CA	Ratio A : Na	BF	PRA
Control						
1	C	125	46	3.8	1.7	8.5
2	+ S	130	63	5.6	2.4	
3	C	115	29	3.3	0.9	
4	+ S	120	56	3.7	1.3	
Indomethacin ($5mgkg^{-1}$)						
5	C	120	69	4.9	1.7	6.7
6	+ S	120	47	4.5	1.1	
7	C	125	80	4.3	1.5	12.5
8	+ S	125	50	4.0	0.6	
Captopril (25mg) (+ Indomethacin)						
9	C	105	48	6.4	2.5	19.3
10	+ S	105	53	3.6	2.6	
11	C	110	87	3.6	2.1	
12	+ S	110	74	3.9	2.1	

Blood pH and blood gas measurements (n=6) -:

pH = 7.27 ± 0.03

pCO_2 = 42.3 ± 3.20

pO_2 = 271.0 ± 7.8

DOG 46. 25kg, M

S	DRUG Test	SBP	CA	Ratio A : Na	BF
Control					
1	C	85	8	5.2	2.4
2	+ S	100	356	2.5	1.9
3	C	85	92	3.4	2.2
4	+ S	100	423	3.2	2.1
Indomethacin ($5mgkg^{-1}$)					
5	C	90	52	3.7	1.8
6	+ S	100	382	2.6	1.8
7	C	70			0.9
8	+ S	85			1.0
Captopril (25mg) (+ Indomethacin)					
9	C	65	40	2.6	1.8
10	+ S	85	272	2.8	2.2
11	C	65	32	2.9	1.9
12	+ S	85	240	3.0	1.8

Blood pH and blood gas measurements (n=4) -:

pH = 7.29 ± 0.06

pCO_2 = 38.8 ± 1.10

pO_2 = 196.3 ± 12.5

DOG 47. 13kg, M

S	DRUG Test	SBP	CA	Ratio A : Na	BF
Control					
1	C	85	85	7.4	1.8
2	+ S	90	296	4.2	2.1
3	C	85	94	7.3	1.3
4	+ S	100	361	5.0	1.5
Indomethacin ($5mgkg^{-1}$)					
5	C	75	135	7.0	0.9
6	+ S	90	387	6.3	1.3
7	C	85	159	8.4	0.8
8	+ S	92	266	6.1	0.8
Captopril (25mg) (+ Indomethacin)					
9	C	90	55	6.3	1.1
10	+ S	90	812	6.9	1.0
11	C	90	51	8.3	0.7
12	+ S	90	62	6.4	0.7

Blood pH and blood gas measurements (n=4) -:

pH = 7.47 ± 0.02

pCO_2 = 30.5 ± 3.10

pO_2 = 325.5 ± 46.0

DOG 48. 23kg, M

S	DRUG	Cortisol conc.	BF
	Control		
1		13.1	1.5
	ACTH (100ug)		
2	+10 min	13.1	2.2
3	+20 min	14.8	1.8
4	+30 min	13.8	1.7
5	+40 min	16.2	1.6

Blood pH and blood gas measurements (n=5) -:

pH = 7.33 ± 0.04

pCO_2 = 39.2 ± 4.40

pO_2 = 232.0 ± 6.8

DOG 49. 22kg, M

S	DRUG	CA	Ratio A : Na	BF	Cortisol conc.
	Control				
1		86	4.16	4.4	4.21
2		46	4.76	2.8	
3		32	4.75	3.0	
4		45	4.45	3.4	
	ACTH (100ug)				
5	+10 min	112	4.76	4.3	3.96
6	+20 min	111	4.84	3.9	5.05
7	+30 min	111	4.43	3.8	5.37
8	+40 min	141	4.70	3.7	6.20

Blood pH and blood gas measurements (n=7) -:

pH = 7.39 ± 0.03

pCO_2 = 40.1 ± 1.40

pO_2 = 144.0 ± 14.5

DOG 50. 27kg, F (see table 2.10)

Blood pH and blood gas measurements (n=5) -:
pH = 7.38 ± 0.04
pCO_2 = 44.0 ± 3.50
pO_2 = 202.0 ± 42.9

DOG 51. 23kg, M

S	DRUG	CA	Ratio A : Na	BF	Cortisol conc.
	Control				
1		209	6.30		
2		88	4.80	2.3	4.02
3		93	5.10		
	ACTH (100ug)				
5	+10 min	130	6.30	2.8	4.62
6	+20 min	202	5.20	2.3	6.69
7	+30 min	112	5.30	3.1	4.91
8	+40 min			3.0	

Blood pH and blood gas measurements (n=5) -:
pH = 7.37 ± 0.01
pCO_2 = 36.8 ± 1.40
pO_2 = 183.5 ± 15.7

DOG 52. 22kg, M

DRUG S	Test	SBP	CA	Ratio A : Na	BF
Control					
1	C	100	482	4.8	1.9
2	+ S	100	575	4.8	1.6
3	C	85	158	7.3	0.6
4	+ S	85	453	4.4	2.2
Flurbiprofen ($5mgkg^{-1}$)					
5	C	65	72	6.0	1.2
6	+ S	70	253	4.7	1.1
7	C	70	30	6.0	0.9
8	+ S	70	78	8.3	0.8
Captopril (25mg) (+ Flurbiprofen)					
9	C	80	55	3.0	2.5
10	+ S	80	68	4.7	2.3
11	C	80	43	4.5	2.1
12	+ S	85	88	4.5	2.0

Blood pH and blood gas measurements (n=6) -:

pH = 7.39 ± 0.02

pCO_2 = 34.8 ± 2.50

pO_2 = 259.0 ± 16.2

CAT 1 2.6kg, F

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
Control							
1	0	140	70	32	2.8	1.7	56
2	1-2	100	120	82	2.3	2.1	61
3	5-6	100	120	58	2.9	1.9	16
4	9-10	100	75	72	3.1	1.4	74
5	0	140	70	78	2.8	2.0	40
6	1-2	100	95	120	2.3	2.0	
7	5-6	100	95	134	2.6	1.5	32
8	9-10	100	95	112	2.5	1.3	
Captopril (25mg)							
9	0	140	50	15	3.7	1.3	24
10	1-2	100				1.5	24
11	5-6	100				1.5	40
12	9-10	100				1.4	16
13	0	140	50	75	1.4	1.5	32
14	1-2	100	70	91	1.4	1.5	
15	5-6	100	70	35	1.2	1.5	40
16	9-10	100	60	41	1.4	1.5	32
AII ($5ngmin^{-1}$)							
17	0	140	65	444	1.8	1.5	
18	1-2	100	100			1.8	
19	5-6	100	100	511	1.9	1.4	
20	9-10	100	75	498	1.8	1.6	
21	0	140	70	284	2.2	2.2	
22	1-2	100	110	464	1.2	2.5	
23	5-6	100	110	594	1.1	1.9	
24	9-10	100	75			1.7	

Blood pH and blood gas measurements (n=8) -:

pH = 7.34 ± 0.03

pCO_2 = 30.0 ± 2.20

pO_2 = 234.6 ± 29.7

CAT 2 3.0kg, F

S	DRUG T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
	Control						
1	0	120	55	40	4.5	0.7	17
2	1-2	80	90	136	3.6	0.9	19
3	5-6	80	90	84	4.8	0.7	18
4	9-10	80	90	92	5.3	0.7	19
5	0	120		98	5.8	0.9	24
6	1-2	80		200	6.9	1.2	12
7	5-6	80					26
8	9-10	80					
	Captopril (25mg)						
9	0	120	50	56	2.0	1.7	48
10	1-2	80	60	69	1.8	1.8	16
11	5-6	80	60	70	3.8	1.7	18
12	9-10	80	60	61	3.1	1.5	12
13	0	120		96	3.0	1.5	12
14	1-2	80		113	3.4	1.6	21
15	5-6	80		125	2.4	1.6	8
16	9-10	80		144	1.9	1.6	12
	AII (5ngmin^{-1})						
17	0	120	80	100	6.9	1.6	
18	1-2	80	80	166	2.2	1.8	
19	5-6	80	80	186	1.7	1.5	
20	9-10	80	80	206	1.8	1.5	
21	0	120		209	1.9	1.2	
22	1-2	80		258	1.4	1.4	
23	5-6	80		264	1.4	1.4	
24	9-10	80		309	1.1	1.2	

Blood pH and blood gas measurements (n=9) -:

pH = 7.40 ± 0.04

pCO_2 = 25.8 ± 4.10

pO_2 = 243.0 ± 28.2

CAT 3 2.7kg, F

S	DRUG	T +BRS	CPP	SBP	CA	Ratio A : NA	BF	PRA
	Control							
1		0	130	100	44	2.7	0.9	4
2		1-2	90	120	90	2.7	0.9	8
3		5-6	90	120	184	1.2	0.9	24
4		9-10	90	105	116	2.1	0.7	16
5		0	130	70				8
6		1-2	90	110				
7		5-6	90	110				
8		9-10	90	90				20
	Captopril (25mg)							
9		0	130	80	46	3.1	0.8	3
10		1-2	90	135	59	3.1	1.3	
11		5-6	90	135	59	4.8	1.1	5
12		9-10	90	120	53	4.8	0.8	8
13		0	130	90	57	6.0	0.5	
14		1-2	90	130	93	6.7	0.6	
15		5-6	90	130	71	6.0	0.5	
16		9-10	90	100	44	6.0	0.4	
	AII ($5ngmin^{-1}$)							
17		0	130	100	80	3.9	0.7	
18		1-2	90	140	123	1.4	1.0	
19		5-6	90	140	110	1.4	0.9	
20		9-10	90	135	136	1.4	1.0	
21		0	130	100	90	2.7	0.8	
22		1-2	90	130	123	1.4	1.0	
23		5-6	90	130	122	1.8	0.9	
24		9-10	90	110	60	2.6	0.5	

Blood pH and blood gas measurements (n=8) -:

$pH = 7.42 \pm 0.03$

$pCO_2 = 29.0 \pm 2.00$

$pO_2 = 249.0 \pm 25.1$

Appendix 2

Drugs used and suppliers

Sodium pentobarbitone (May and Baker)

Heparin (Weddel Pharmaceuticals)

Captopril (Squibb)

Angiotensin I (acetate salt) (Sigma)

Angiotensin II (acetate salt) (Sigma)

Saralasin ([Sar^1,Ala^8]-Angiotensin II) (Sigma)

ACTH (Synacthen) (Ciba)

Cycloheximide (Sigma)

Naloxone (hydrochloride) (Sigma)

Indomethacin (Sigma)

Flurbiprofen (Boots)

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Modulation by the renin–angiotensin system of the baroreceptor reflex response of the adrenal medulla in the dog

By Margaret R. MacLean and A. Ungar. *Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh 8*

Feuerstein, Boonaviroj & Gutman (1977) showed that blockade of the renin–angiotensin system in the cat inhibits the release of catecholamines (CA) from the adrenal medulla in response to haemorrhage, and concluded that sympathetic activation of renin release from the kidney mediates the adrenal response to haemorrhage, by a central action of angiotensin.

We have carried out experiments on six dogs, anaesthetized with pentobarbitone (30 mg kg^{-1}). Both carotid bifurcations were prepared, and the venous outflow of the left adrenal gland collected as described by Critchley, Ellis, Henderson & Ungar (1982). Both cervical vagosympathetic trunks were cut. Baroreceptor tests were performed by lowering carotid sinus pressure by 40 mmHg for 10 min. CA output (adrenaline + noradrenaline) was estimated spectrophotofluorometrically in adrenal venous blood. Plasma renin activity (PRA) was estimated in arterial blood samples. It is expressed in units of ng angiotensin per ml plasma per hour.

Baroreceptor tests raised CA output from $26{\cdot}4 \pm 4{\cdot}3$ pmol min^{-1} kg^{-1} by $63{\cdot}6 \pm 12{\cdot}4$ pmol min^{-1} kg^{-1}. PRA, initially $48{\cdot}7 \pm 10{\cdot}5$ units, did not rise during baroreceptor tests. After injection of the angiotensin converting enzyme inhibitor captopril (1–1·3 mg kg^{-1} i.v.), both resting and stimulated release of CA was reduced. Baroreceptor tests now raised CA output from $16{\cdot}6 \pm 3{\cdot}3$ pmol min^{-1} kg^{-1} by $12{\cdot}6 \pm 5{\cdot}5$ pmol min^{-1} kg^{-1}.

Angiotensin II (AII) was now infused (50–200 pg min^{-1} kg^{-1}). This did not affect arterial pressure, but restored the resting output of CA to $25{\cdot}2 \pm 7{\cdot}3$ pmol min^{-1} kg^{-1}. Baroreceptor tests during AII infusion raised CA output by $32{\cdot}0 \pm 9{\cdot}8$ pmol min^{-1} kg^{-1}. Cycloheximide (50 mg kg^{-1} i.v.), given after captopril, lowered the resting output of CA to $11{\cdot}0 \pm 3{\cdot}6$ pmol min^{-1} kg^{-1}. Cycloheximide abolished the adrenal response to baroreceptor tests during AII infusion, although the pressor response to baroreceptor tests was not impaired. In the absence of captopril, this dose of cycloheximide does not abolish the adrenal response to baroreceptor tests, but blocks the release of corticosteroids and CA in response to corticotrophin (Critchley *et al.* 1982).

We confirm the finding of Feuerstein *et al.* (1977) that the integrity of the renin–angiotensin system is necessary for the reflex response of the adrenal medulla. Our results do not support the interpretation that the release of renin mediates the response, but suggest rather that a minimum circulating level of AII is needed for the adrenal gland to respond to the reflex stimulus. The action of cycloheximide suggests that AII may act by releasing corticosteroids.

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Modulation by angiotensin of the output of catecholamines from the adrenal medulla and of its response to splanchnic nerve stimulation in the dog

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We have previously shown that the angiotensin converting enzyme inhibitor captopril inhibits the reflex release of catecholamines from the adrenal medulla in response to the lowering of carotid sinus pressure, and that the response can be restored by the infusion of angiotensin II (AII) or of ACTH (MacLean & Ungar, 1984). These results suggested a permissive action of AII on the adrenal gland, but did not exclude an action within the central nervous system, as postulated by Feuerstein, Boonaviroj & Gutman (1977).

In eleven dogs, anaesthetized with pentobarbitone (30 mg kg^{-1}), the peripheral cut end of the left splanchnic nerve was stimulated electrically, and the venous outflow of the left adrenal gland collected for the estimation of catecholamines by HPLC and of cortisol by RIA.

Captopril (1 mg kg^{-1} I.V.) reduced the mean resting output of total catecholamines (adrenaline + noradrenaline) from 120 ± 37 to 104 ± 27 pmol min^{-1} kg^{-1}. The mean incremental release evoked by splanchnic nerve stimulation at 10 p.p.s. was reduced from 100 ± 21 to 40 ± 16 pmol min^{-1} kg^{-1} ($P < 0{\cdot}01$). A similar reduction in resting and stimulated output was found during the infusion of the angiotensin antagonist saralasin (10 μg min^{-1} kg^{-1}). In five dogs, after captopril, infusion of AII (10–100 ng min^{-1} kg^{-1}) raised both the resting and the stimulated catecholamines, without affecting arterial pressure. After the injection of captopril, the mean cortisol output of the left adrenal gland rose from 320 ± 40 to 480 ± 80 ng min^{-1} kg^{-1} ($P < 0{\cdot}05$).

We conclude that AII facilitates the response of the adrenal medulla to sympathetic stimulation. The rise in cortisol output after captopril makes it unlikely that the main action of AII on the medulla involves the release of corticosteroids (MacLean & Ungar, 1984).

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